HUMAN MILK

YIELD, PROXIMATE PRINCIPLES AND INORGANIC CONSTITUENTS

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YIELD, PROXIMATE PRINCIPLES AND INORGANIC CONSTITUENTS

BY

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PREFACE

THE preparation of this communication was undertaken while I was on the staff of the Commonwealth Bureau of Animal Nutrition and was continued and completed after I had come to this Institute. During its preparation a report was issued by Kon and Mawson (Medical Research Council Special Report Series, No. 269, 1950) which reviewed the relevant work on the vitamin content of human milk and contributed new information on vitamin content and general composition. It did not seem profitable to attempt to duplicate that work and so the present communication is confined to a consideration of the composition of human milk in terms of proximate principles and minerals, along with a section on yield. Readers who require information on vitamin content are referred to Kon and Mawson's report.

The bibliography given here is not exhaustive, but includes references to all the papers which I have been able to find which had, in my opinion, something of value to contribute to the knowledge of human milk composition.

Wherever the original experimental data were given in detail by an author I have computed the means afresh and, in addition, the standard errors. In the following pages, all measures of dispersion are standard errors, except where specifically stated to be standard deviations (S.D.) or ranges. The quotation of a range or of a simple mean implies that it was not possible to derive any valid measure of dispersion from the data given. In order to cast the findings of different workers in comparable form I frequently had to convert the basic data from per 100 g. to per 100 ml. basis, and make minor adjustments such as the elimination of colostral values from a predominantly mature milk series. The result of these changes is that the mean values given here are not always identical with those given in the original papers.

I have also, from time to time, made a statistical analysis although the experiments were not designed to make such analysis valid. My use of these forms has simply been to present a brief quantitative description of the results of an investigation, and any significance level or probability must be viewed with caution.

I wish to record my thanks to Mr. P. C. Jowsey who prepared all the diagrams for publication, and to Mr. W. Godden for his patient checking of my figures and calculations and for many valuable suggestions. I also wish to record my indebtedness to Dr. I. Leitch, on whose suggestion this work was undertaken, for constant encouragement and help, and to Professor R. C. Garry for his continued interest during the later stages of this work.

S. D. Morrison.

Institute of Physiology,
Glasgow University.
July, 1951.

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by

S.D. Morrison

Errata

p.2, text line 12

For Ström read Newton and Newton

p.11, text line 7

After "produced" insert "at a nursing"

p.11, fourth line from foot

After "Ström (1948) recorded the" insert "total"

p.11, second line from foot

For 308 read 380; For 1900 read 1090

p.13, text line 1

For Winikoff read Nims

p.26, Table 4, column 11, line 13

For 0.154 read 1.154

p.27, Table 5, column 6, last line

For 0.07 read 0.97

p.52, line 23

For "fed on" read "whose mothers gave to the milk bank"

p.52, line 28

After "consistent" delete rest of sentence

Addendum

Footnote to Fig. 1. The volume recorded by Deem appears to be for the one breast from which milk was expressed.

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HUMAN MILK

VOLUME OF MILK

The volume of milk produced is known to differ between breasts, and to vary from nursing to nursing and from day to day. The data on which this knowledge is based are scanty but suffice to give the trends of the pattern.

There are two methods of measuring the quantity of milk yielded and neither is completely satisfactory. The child is weighed before and after nursing and the difference is taken as the weight of the milk. The insensible weight loss of the infant during nursing is not commonly taken into account, but is probably of little moment. The other method is to extract the milk manually or by breast pump and weigh it. Manual expression is recommended by Macy and her co-workers and a special technique has been described (Davies, 1945), which is claimed to give more complete expression of the milk than any of the mechanical pumps. Volumes obtained by this method are illustrated by the data summarised by Kaucher *et al.* (1945) and Roderuck *et al.* (1946*a*) (see pp. 7, 10). To compare the two methods, weighing the infant and manual expression, Deem (1931) expressed the milk manually from one breast and allowed the infant to suckle the other ; the weight of the milk obtained by the infant was, in general, greater than that obtained manually. Deem did not give the values obtained for manual expression, as she assumed that the amount obtained by the infant was the true yield. On the other hand, Pierangeli and Escudero (1939) appear to have allowed the child to suckle to satiety * and then to have extracted

* The position is not clear and correspondence with Dr. Pierangeli leaves some doubt. The only yields recorded by this group of workers are those for colostrum by Escudero and Esquef (1944), who measured the volume at one milking and record daily volume as 5 times that measured. All other data are for composition of the " surplus " milk drawn once or twice daily and analysed as a control of quality. This suggests that, except for mothers whose infants had been weaned or had died, the infants had already suckled when the surplus was drawn and the samples represented after milk. This possibility should be remembered, especially when Table 12 is considered.
We are grateful to Dr. Pierangeli for information on other details.

milk with an electrical suction apparatus, so that the amount obtained by the infant is not necessarily the maximum obtainable.

Variation between Breasts

This variation was investigated by Macy *et al.* (1930, 1931) on 4 women for a total of 7 days. The variation in volume was considerable and in 5 out of the 7 trials the yield of one breast exceeded that of the other for each of the 6 nursings in the day. The mean total daily volumes in ml. for the right and left breasts for the 7 trials were :

R . .	1438*	1144*	569*	1169*	1681*	310	244
L . .	1279	1490	691	1493	1451	366	288

* Significant difference when considered in terms of individual nursings.

Deem in her study does not appear to have made any change-over experiment to find if the differences were in fact due to method of extraction or to actual differences between breasts. More recent work (Ström, 1948) has shown the great effect of method of extraction on the yield of milk. A greater amount of milk can be obtained by pump from one gland if the other is simultaneously suckled, and the greatest amount can be obtained by pump if pitocin is first given, intramuscularly.

It would seem reasonable to suppose that the two breasts, if subjected to the same stimulus, would either yield approximately the same amounts, or show a systematic difference indicating different efficiencies of the glands. The fact, demonstrated by Brown *et al.* (1932), that the percentage composition of the milk from the two breasts does not differ appreciably, does not indicate different efficiencies (see also p. 19). It seems possible that the differences observed between the yields from the two glands are produced by differences in external stimulus, that is, by differences in the method of manual expression, differences between manual expression and suckling, or even differences in the infant's enthusiasm during the course of nursing, but Vincent and Vial's (1933*c*) findings do not support this idea. When both breasts were expressed manually at the same time one breast in almost all subjects gave a higher yield at all milkings than the other. The

fat content did not differ. Macy *et al.* (1930) found evidence that the greater volume is secreted by the larger breast.

Diurnal Variation

Diurnal variation is important in sampling and gives part of the picture of the physiology of milk secretion. The volume per nursing plotted against the time of nursing throughout the day, derived from the data of Engel (1909), Denis and Talbot (1919), Macy *et al.* (1930, 1931), Deem (1931) and Winikoff (1944), is shown in Fig. 1. With the exception of Macy's work, all these curves indicate that volume is at a maximum in the early morning and decreases towards evening. A conclusion that might be drawn from Deem's data is that the interval given to the gland to secrete influences the volume obtained at a subsequent nursing. This may be clearly seen by comparing the volume produced after an 8-hour interval (overnight) with that produced after 4-hour intervals during the day. If, however, the volume produced on sampling is reduced to the apparent volume produced per hour over the preceding period it will be seen that the rate of production is approximately the same throughout the whole 24 hours. Deem's is the only curve which levels off so neatly during the day and evening ; Denis and Talbot's and Engel's become irregular in the evening. Both sets of data, on the basis of assumed rate per hour, suggest a low rate of secretion during the night, and Denis and Talbot's have a definite maximum at the 9 a.m. and noon samplings. The curve from Engel's data shows a considerable rise in the late evening after passing through a minimum at about 6 p.m. The two exceptions to the general pattern are Macy's (1930) data, which show a considerable rise in the late evening reaching almost to the early morning maximum followed by another fall, and Macy's (1931) data, which show a minimum occurring about mid-morning followed by a maximum soon after noon. Her results were based on only 3 women compared with 5 in Deem's study and 8 in Engel's, but Macy made observations at 4-hour intervals throughout the day and night, and the others only from 6 a.m. to 11 p.m.

Winikoff's data are for colostrum at 7 and 9 days and so are not directly comparable with the others.

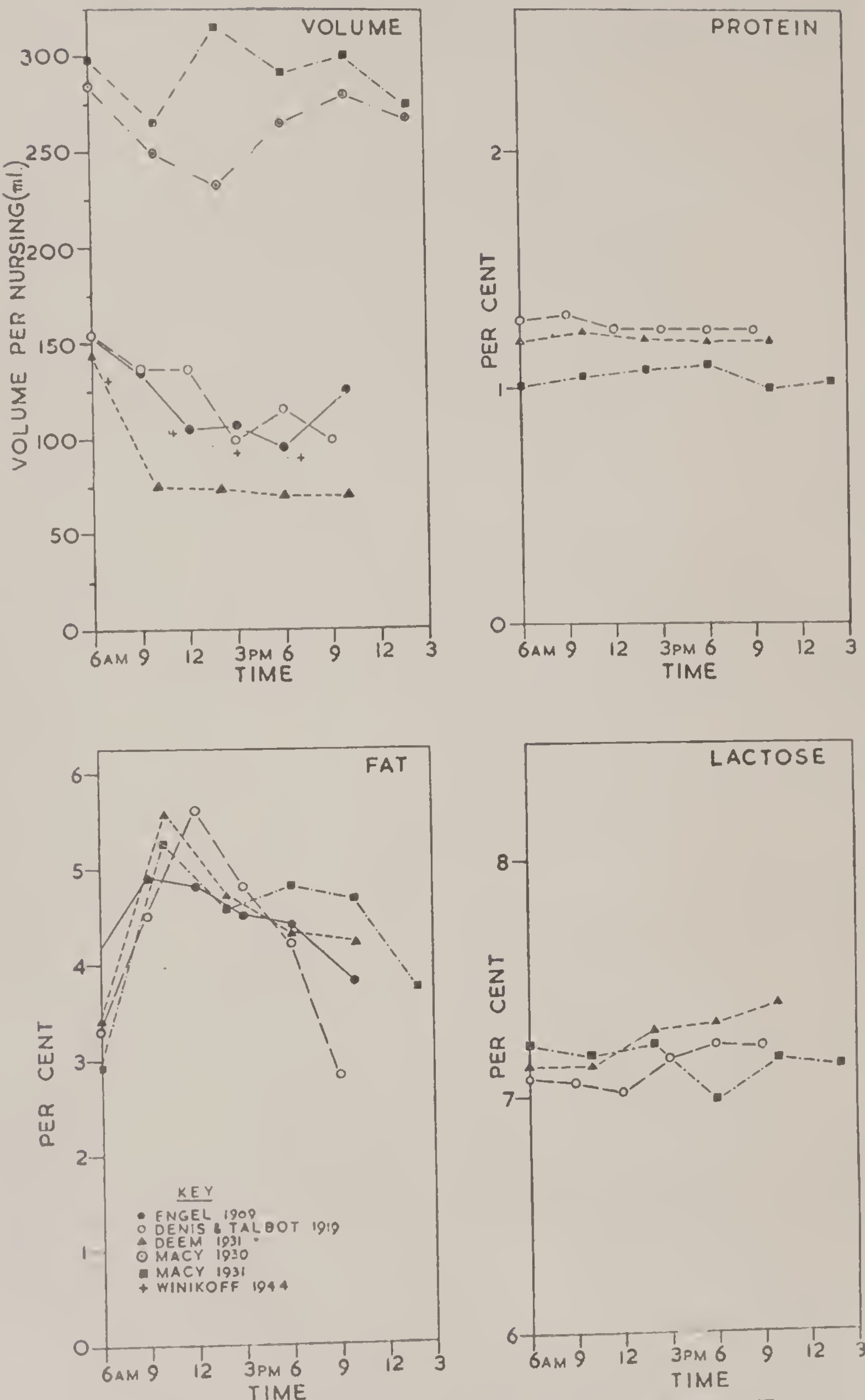


FIG. 1 DIURNAL VARIATION OF VOLUME AND OF PROTEIN, FAT AND LACTOSE CONTENTS IN G. PER CENT.

Vincent and Vial (1932, 1933*a*) examined diurnal variation in the same women for 11 consecutive days and found the cyclical variation to be continuously maintained.

Macy (1930) investigated the capacity of the breast as shown by volumes obtained after long intervals and when these data are reduced to apparent yield per hour the volume produced over the long intervals, 13 and 16 hours, is greater than the average production over the whole lactation but less than that produced on the day noted as that on which the highest yield was given.

In a series of 70-day studies giving 1680 results, Jaso (1949), expressing his results as hourly secretion rates, found a plateau of maximum secretion between 6 a.m. and 11 p.m. with a not very pronounced " point " maximum at about 11 a.m. A nocturnal minimum occurred about 3 a.m. His diagram, which shows volume per nursing over the day, with an 8-hour night interval and 4-hour intervals during the day, follows the general pattern, showing a drop in yield, per nursing, from morning to night. His explanation of the diurnal rhythm is not that there is an increased rate of secretion overnight but that the milk which was not entirely removed during the day nursings contributes towards the morning total together with that secreted overnight. He observed also, that where there was abundant secretion, the maximum yield in terms of yield per hour was in the morning, and when secretion was scanty the maximum came in the late afternoon or evening. It is difficult, without a full explanation of his sampling technique and methods of computation, to judge how this fits with his theory of carry-over from one day to the next.

There is not enough evidence to justify the statement that yield varies only with the interval between samplings. It seems likely that diurnal variation in yield at stated intervals is the result of at least two causes, of which one may be the length of interval and the other some cyclical influence of unknown origin, but probably connected with strength of stimulus. Even with manual or mechanical expression stimulation is almost bound to vary. Nor must the state of mind of the subject be ignored, as it may considerably influence the let-down reflex (Newton and Newton, 1948).

Variation with Stage of Lactation

As far as can be traced the only major work directed specifically towards elucidating this variation is some detailed and thorough work done at the beginning of the century by Schlossmann (1900,1902) and Peters (1902), but their subjects were professional wet nurses. It is not always clear what is the total yield of the women and what is the intake of each child. The volumes in these investigations were found by weighing the infants. In much of the more recent work also, the subjects used were wet nurses, for instance, the women studied by Macy and her team in their earlier work ; and even the milk donors of Escudero and his team were women with more than ordinary milk yields. These researches show that in a single lactation a high yield may be maintained for long periods, over a year, but they do not show what curve of yield is to be expected from the average mother. The daily yields found by Schlossmann for his 3 main subjects show great day-to-day variations. In the 2 subjects for whom the greatest number of observations was made, maximum yield appeared to occur at about 100 days and afterwards the yield tended to decline, though with irregular periods of recovery. At about 20 days, the yields of 2 of the subjects were about a litre and at the maximum one of these averaged about 2200 ml. The third gave about 400 ml. at 20 days and about 1300 at 100 days. The findings of Laurentius (1911), Brodsky (1914) and Kollman (1927) were much the same as those of Schlossmann with a plateau of maximum yield beginning between 40 and 100 days and ending between 80 and 200 days. Great individual variation occurred in the length of the plateau, the time at which it occurred and the maximum yields obtained.

Roderuck *et al.* (1946*a*, *b*), in the course of a study of vitamins in milk, obtained records of the milk flow of 7 women in the first 10 days *post partum*, and the graphs and mean values are shown in Fig. 2. For comparison, the volumes estimated by Escudero and Esquef (1944) as 5 times the yield of one morning milking were 170, 290 and 415 ml. on the 2nd, 3rd and 4th days. These values represent the average yields of 24, 57 and 20 donors. The form of the graph is in agreement with common experience and expectation. It can be seen that the curve has flattened out by the end of

the 10-day period. Previous work by Nims *et al.* (1932*b*) gave average values for volume over 4-day periods at the 8th, 24th, 32nd and 56th weeks of lactation (Fig. 3). These suggest that the peak

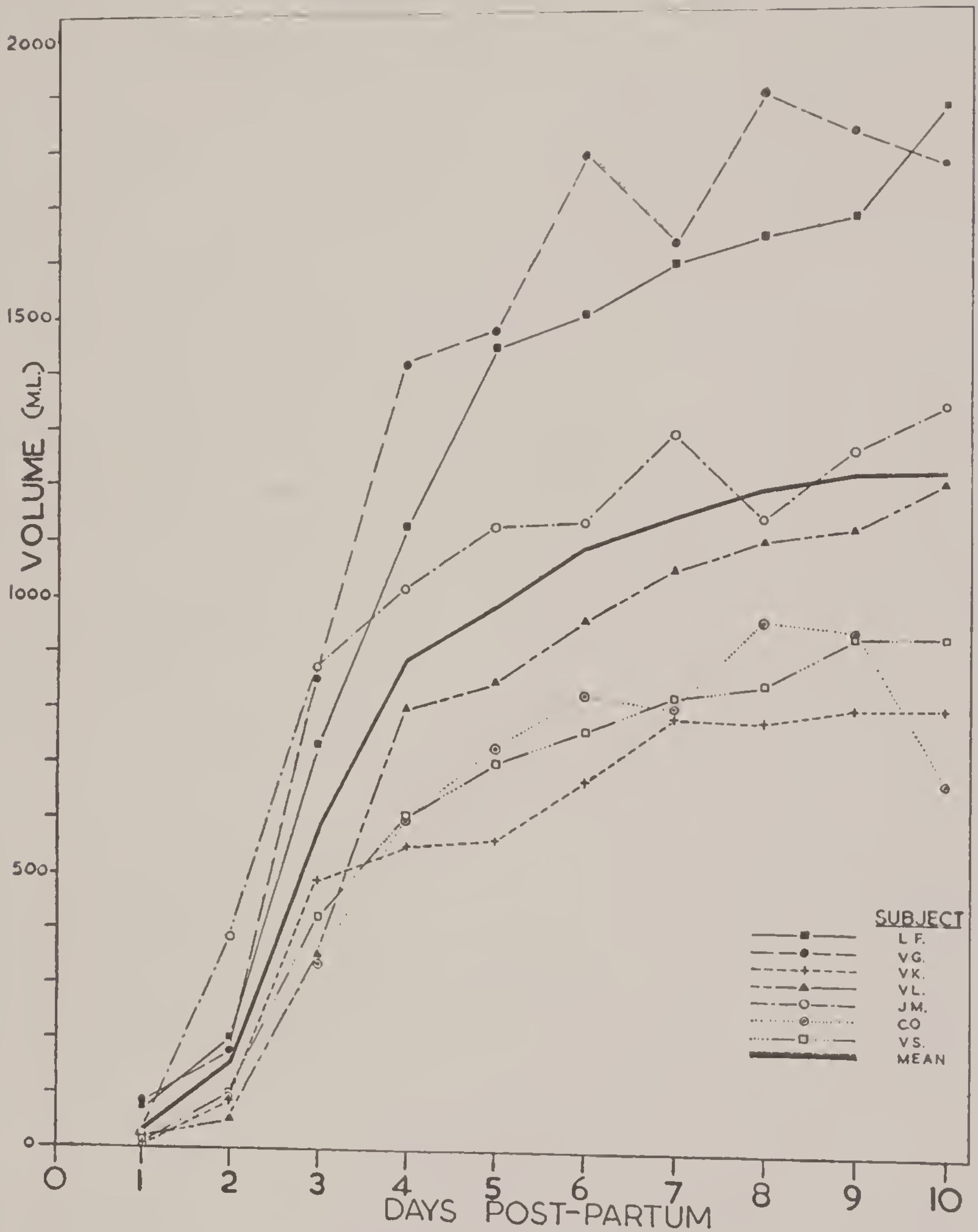


FIG. 2 VARIATION OF VOLUME DURING FIRST 10 DAYS *Post Partum* (Roderuck *et al.*, 1946*a*, *b*.)

volume lies somewhere between the 20th and 32nd weeks of lactation. This is later than would be expected from Schlossmann's and Brodsky's subjects. Macy *et al.* (1930) recorded peak production for 3 women at 48, 135 and 186 days. It seems

unlikely that the shape of the colostrum curve, almost horizontal on approaching the 10-day mark,* is a true indication of the general trend after that time, but it is generally held that the volume

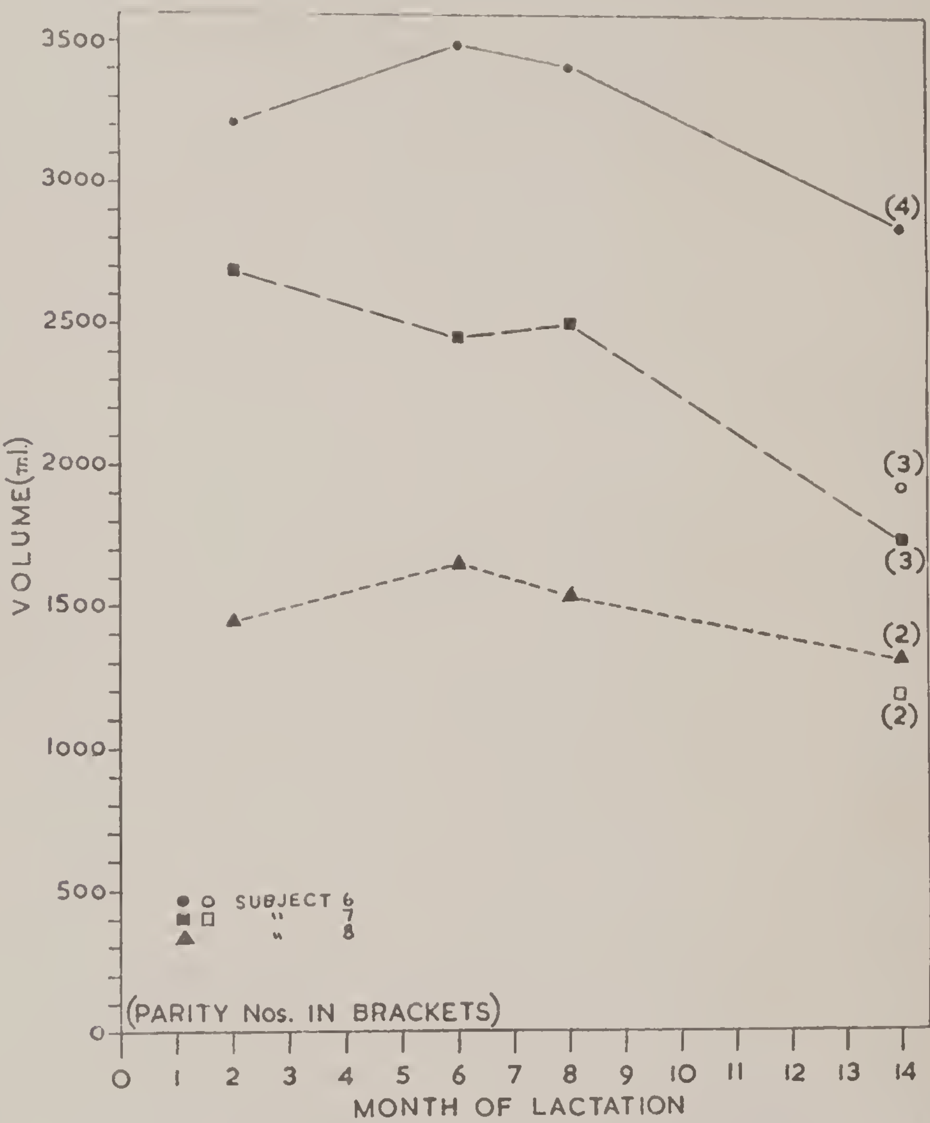


FIG. 3 VARIATION OF VOLUME WITH STAGE OF LACTATION AND PARITY (Nims *et al.*, 1932*a*, *b*.)

at the end of the colostral period may far exceed the flow maintained afterwards, and this is true of volume records made by Roderuck *et al.* (1946*a*, *b*) at a later stage in lactation.

* The end of the colostrum period is indefinite and has been arbitrarily fixed at from 3 to 20 days. In this review the general assumption is that it ends at 10 days since, by that time, the main characteristics of mature milk have been established. Colostral corpuscles disappear after 3 to 7 days.

Daily milk yield for 5-day periods was recorded for different women, including 5 of those whose colostral output was recorded, at 70, 160 and 300 days. Yields were fairly low, only 1 woman reaching 1000 ml. on 1 day. In 10 out of the thirteen 5-day periods recorded the yields increased from beginning to end of the period. Here, as previously, manual expression was used and it would appear that the experimental procedure, or the renewal of it, or causes associated with it, may have effectively reduced the flow of milk, which tended to rise again to normal as the mother got used to the procedure. But it is to be noted that although the trend may have been towards normal flow, there is no indication that after 5 days' habituation normal flow was actually re-established. Data for 4 of the Detroit subjects (Roderuck *et al.*, 1946; Munks *et al.*, 1947) for whom most records were obtained are graphed (Fig. 4) to illustrate this effect. In view of the order of mid-lactation volumes given by other workers, and those previously found by Macy and her co-workers, the yields recorded in this study seem exceptionally small, the yield at 12 to 15 weeks being only half of that at 10 days. Inadequacy of the energy value of the home diet cannot explain the fall since it was usually of the order of 3000 Cal. (Kaucher *et al.*, 1946), and the differences in intakes of protein and vitamins in the hospital (where the colostrum yield was recorded) and at home were small. If in fact the manual expression of milk or the associated experimental procedure tends to reduce the flow, then it is possible that the milk flow recorded for the first 10 days should also be higher, which would make the fall between colostrum and mature milk volumes even greater. It may be, however, that experimental procedure combines with home environment to produce this reduction of yield.

Macy (1930, 1945) claims that milk flow adjusts itself to the demand, but it can hardly be claimed that a 12- to 15- week-old baby demands only 500 ml. of milk. Kaufmann and Bickel (1931) record no higher consumption by infants than 500 ml. although considerable quantities were removed after suckling, but their observations extended only to 10 days after birth. Wallgren (1944-45) in a study of 363 infants records a rise from about 490 g. at 2 weeks of age in both sexes, to maxima of about 750 g. for girls

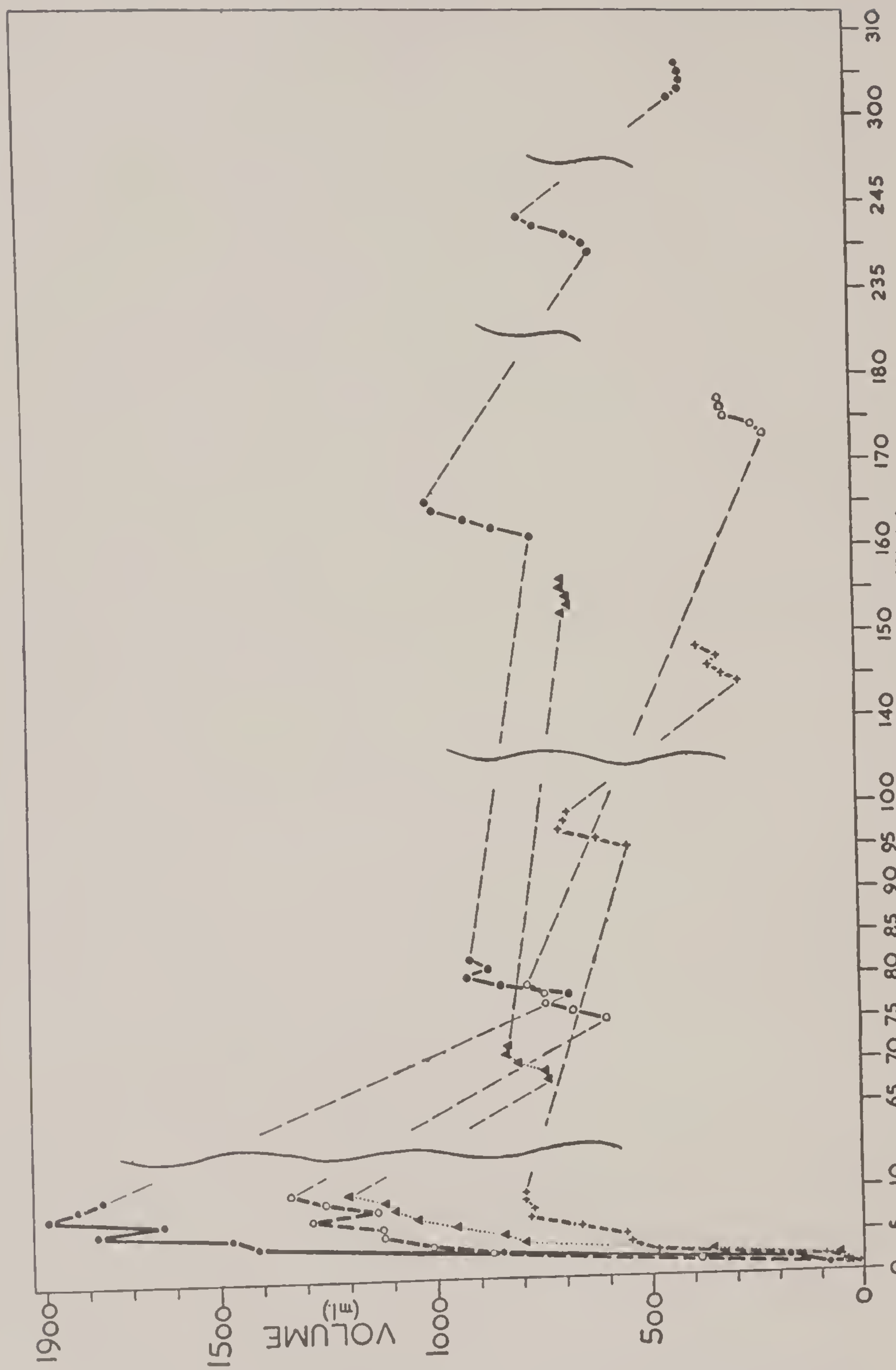


FIG. 4 VARIATION OF VOLUME WITH STAGE OF LACTATION
(Roderuck *et al.*, 1946*a*, *b*.)

and 820 g. for boys at 4 months, with a plateau between 4 and 6 months. On the other hand, Schoedel (1934), in a study of 239 infants, records consumption as rising from 520 g. on the 11th day to 885 g. during the 5th month in a private nursing home and from 435 to 795 g. in a maternity home. From the data of Smith and Merritt (1922) a regression equation relating weight of milk produced to weight and age of normal infant can be derived :

$$z = -0{\cdot}45 + (0{\cdot}506 \pm 0{\cdot}099)\,x - (0{\cdot}189 \pm 0{\cdot}056)y$$

where z is the weight of milk produced in ounces, x the weight of the child in pounds and y the age of the child in weeks. This suggests that the weight of the child may be of greater importance to milk yield than its age. The effect of weight of the child on the milk yield was also shown by Wardlaw and Dart (1932). On this showing, then, we are left to conclude that the mid-lactation volumes recorded by Macy and her collaborators for these ordinary women are abnormally low, and indeed that any occasional estimate of yield may be fallaciously low.

All values for daily volume of milk for which the stage of lactation is known have been plotted against time in Fig. 5. This represents chiefly data for wet nurses and must be interpreted with caution in relation to the lactation performance of average mothers.

Variation with Parity

The data available on this variation are few and inconclusive. Nims *et al.* (1932*a*, *b*) for two of their subjects gave the average yield at 56 weeks for two consecutive lactations. In both subjects the yield in the earlier lactation was only about 65 per cent. of that in the later (Fig. 3). Of Winikoff's (1944) 6 subjects, 3 were primiparae and 3 multiparae (one 2nd, one 3rd and one 5th) and here a general tendency could be seen for the yield in the first pregnancy to be higher than in succeeding pregnancies. Nims's data were from consecutive lactations of the same women and, with such small numbers, are probably preferable to data from different women. Ström (1948) recorded the milk yields from the 2nd to the 7th days of lactation from 228 primiparae and 308 multiparae and found the mean yields to be 1900 ± 24·1 g. and 1243 ± 20·7 g., respectively ; this indicates the same trend

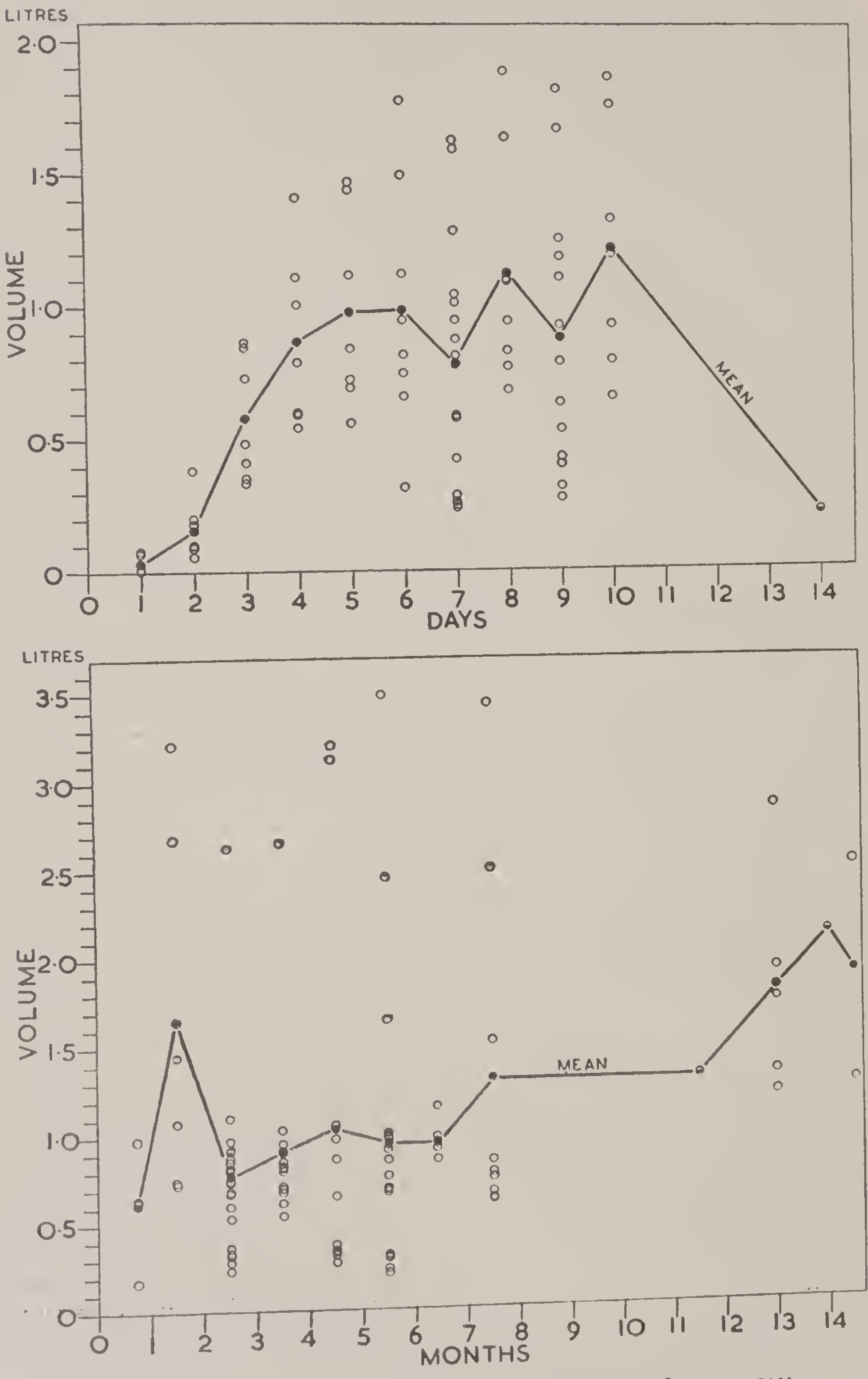


FIG. 5 VARIATION OF VOLUME WITH STAGE OF LACTATION: COLLECTED DATA

as Winikoff's data and the difference is highly significant. Even so, it would be better, before making any decision on the reality of the increase, if we could have a considerable body of data based on successive lactations of the same subjects.

Variation with Age

The only work with a direct bearing on the effect of age is on duration of lactation. The results are confused and inconclusive, but the general impression given is that age has no significant effect. It would be natural to expect that, even if age had no direct effect, it would appear to have some effect because of an association with parity. Ström (1948), however, found an inverse relation between amount of milk (colostrum) and age of mother, and also that this effect was greater in primiparae than in multiparae. If Ström's results be accepted they would go some way towards explaining the variable findings with respect to parity, since the effects of parity and age on milk volume would be antagonistic.

Individual Variation

The variation in volume of milk between individual women is of such magnitude as to dominate completely all other variations in yield of mature milk.

From colostrum data of Roderuck *et al.* (1946*a*, *b*) it is obvious that, at least within these 10 days, the production of milk by individual women follows an individual course. This can be easily seen in Fig. 2, where the individual curves are shown as well as the mean. The graph of Nims's data for volumes in mid-lactation for 3 women also shows clearly an individual course (Fig. 3). The mean yields from each of Deem's (1931) subjects were 1316, 1272, 678, 921, 967 ml., with a standard error of 27·1 (see Table 1).

In Deem's subjects, however, all we know of the stage of lactation is that it was between 4 and 18 weeks. Macy's subjects, although all parallel in stage of lactation, were multiparae and the parities are not known. The parity of Deem's subjects is not stated, but since they were from a home for unmarried mothers it can probably be safely assumed that they were primiparae.

Schlossmann's (1900, 1902) two main subjects, both primiparae, show the same distinction very strikingly. In spite of the large day-to-day variation of as much as 500 ml., the yields overlap only after the peak of production is passed and then scarcely at all. These two subjects were completely different in physical type, but of the same age.

TABLE 1

INDIVIDUAL VARIATIONS IN VOLUME AND COMPOSITION
(Deem, 1931)

Variable \ Subject	A	B	C	D	E	*P*
						S.E. 27·1
Volume ml.	1316	1272	678	921	967	< 0·001
Protein g./100 ml.	1·33	1·06	0·98	1·03	1·30	< 0·001
Fat g./100 ml.	4·2	3·5	3·7	3·8	3·7	< 0·001
Lactose g./100 ml.	7·62	7·38	7·35	7·44	7·41	< 0·001

It seems possible that part of the apparent variation between individuals may be associated with the sources of variation already mentioned, for instance, the probable effect of the weight and enthusiasm of the infant itself. Without a fairly large number of considerable sequences of observations for which the alterable characteristics of the mother and child are known, it is not possible to give even a tentative estimate of what fraction of the total individual variation is due to the individuality of the mother.

Effect of Diet

It is only on Deem's data that this variation can properly be examined. Macy and her co-workers have been especially interested in the effect of diet on milk secretion and composition, but have not published any data on the actual response in volume to changes of diet.

Deem's 5 subjects lived for a week at a time on each of 7 diets in succession. The average daily volumes for each week are given in Table 2. The differences between diets are significant

TABLE 2

EFFECT OF DIET ON VOLUME AND COMPOSITION
(Deem, 1931)

Variable \ Diet	Institution	High-protein	Home	High-protein + Vitamin B	High-sugar	High-fat	Low-protein	*P*
								S.E. 32·2
Volume ml.	927	1037	1002	1091	1064	1076	1017	< 0·05
Protein g./100 ml.	1·16	1·26	1·08	1·19	1·11	1·09	1·08	< 0·001
Fat g./100 ml.	3·8	3·4	3·5	3·7	3·6	4·3	4·2	< 0·001
Lactose g./100 ml.	7·67	7·41	7·53	7·40	7·41	7·28	7·36	< 0·001

but neither so significant nor so great as the differences between women. The differences observed must also be interpreted with caution. The institution diet was the diet on which these women subsisted normally and although they appeared to be healthy and to do a fair amount of physical work, milk yield was less than on any of the other diets. The effect of changing to a high-protein diet would possibly not be seen immediately, and the subsequent effect of the " home " diet would certainly be modified by the carry-over effects of the high-protein diet. Indeed, with such a short experimental period as a week carry-over effects of each diet used to the subsequent one were bound to be present, and it is impossible to resolve them. Thus, all we can say with certainty from this work is that diet may affect volume, but that reasonable changes in diet do not produce differences in the same women comparable with those existing between women independently of diet.

Robinson (1943) reported the finding that, when there was a gradual decline in milk yield, an inverse relationship appeared to

exist between milk yield and amount of muscular exertion. It seems possible that here the decline in yield may be the result of failing appetite, as the women she examined were not, presumably, subjected to any external restriction of food consumption.

Effect of Water Intake

The common belief that fluid intake affects milk yield has little evidence to support it in women. In cows the fluid intake as free water increases with milk yield, and metabolic water rises as a result of increased food intake. The water of faeces and urine and the water of vaporisation also increase at this time, as greater quantities of waste materials have to be excreted in the urine and more waste heat has to be disposed of (Leitch and Thomson, 1944-45). Since there is little work on women it must, for the present, be assumed that the same type of change occurs in lactating women. There are, however, some indications that the parallel is not exact. Macy *et al.* (1930) recorded milk volume and fluid intake for their 3 subjects for about 65 weeks and found no association. Olsen (1941) found that variations in the quantity of liquids ingested by the nursing mother from 600 to 2775 ml. per day had no effect on the breast milk supply as judged by the weight of the milk and the weight increase of the child. This work was done on 13 subjects ; the general plan of each study was that a 3- to 4- day period of "normal" fluid intake (1200 to 1800 ml., varying with the subject) was followed by a similar period of reduced fluid intake (500 to 800 ml., usually 600 ml.), and this was followed by a further similar period of excess fluid intake (2575 to 3175 ml., usually 2775 ml.), followed by a final period of normal intake. The usual length of a complete study was about 14 days. There were two 26-day studies, each containing 10-day periods of 600 ml. fluid intake. In only one case did the milk volume show a definite change, and that a decline, when the period of reduced fluid intake was followed immediately by one of excess intake. In all the other studies the milk yield remained constant, independent of the variation in fluid intake.

Olsen's work gives no information on the general water balance of the mothers or of any bodyweight changes during these

régimes. Lelong *et al.* (1949) reported two similar but greatly extended studies on one subject. The first was of 26 days' duration. The data presented do not allow a satisfactory picture of water balance to be made, but Professor Lelong has kindly supplied further information with which it is possible to deduce roughly by what economies in water metabolism the milk volume was maintained. Throughout the experiment the constant diet supplied about 920 ml. preformed water and about 400 ml. potential water of metabolism. In the first 10 days, during which 1322 ml. water was taken daily as beverages and weight was maintained, the average volume of urine was 932 ml. and of milk water 922 ml. This gives a total intake of 2650 ml. and an output of approximately 1860 ml., leaving 790 ml. as water lost in faeces, respiration and transpiration. In the 11 days in which water drunk was 400 or 500 ml., average 445 ml., milk water was 887 ml., urine volume was 484 ml. and 2·6 kg. weight was lost. Assuming food water to be as before, the total water available from food, drink and weight loss was 2010 ml. daily and output in urine and milk 1370, leaving 640 for faeces, respiration and transpiration. That is to say, there was an economy of approximately 50 per cent. in urine water, which reduces it to about the minimum attainable, and of approximately 20 per cent. in other losses.

The constancy of the milk volume with these different water intakes indicates a strong impetus to milk secretion and a surprising tolerance of water restriction. A limit to the length of the period for which such water restriction can be maintained without effect on the milk yield must of course be reached, but it is not possible from these investigations to know what effect is produced in the end, a gradual decline or a sudden drop of milk yield.

Lelong *et al.* estimated daily the percentage protein, fat and carbohydrate of the milk yielded during their investigation and found no change during either water depletion or excess.

COMPOSITION OF MILK

Nitrogen

What is commonly estimated in milk and often described as total protein is not always just that. The most general practice has been to estimate total N and express it as total protein by multiplying by a constant representing the percentage of N in milk protein. This method applied to cow's milk involves no great error, since the N.P.N. fraction is small, about 5 per cent. of total N, and shows a range of variation of about 25 per cent. around the mean, a range which, with so small an amount, is of little importance. But in human milk the N.P.N. fraction is of considerable magnitude, up to 20 per cent. of the total N, and not at all constant. Thus the computation of protein from total N cannot be considered to be permissible.

Some analysts, particularly the early ones, *e.g.*, Carter and Richmond (1898) and Sikes (1906*b*), estimated the protein by precipitation and weighing, but it is doubtful if this method gives accurate results, and comparison is difficult because few investigators used the same precipitating agent or technique or fully described the techniques used. Certainly Carter and Richmond's values for the " true " protein of mature milk seem high, 1·4 g. per 100 ml., compared with more recent values computed from total N ; on the other hand, so also do Schlossmann's (1900), which were found by estimation, by the Kjeldahl method, of total N.

The Kjeldahl method has itself been subjected to frequent modification, but it will be assumed that these modifications have affected only the speed and convenience of the estimation and have not appreciably altered its accuracy. However this may be, the earlier techniques probably were appreciably less accurate than the more modern.

Before the attempt was made to assess the partition of and the sources of variation in the N of human milk, all available estimates of total N by Kjeldahl which conform to certain criteria were combined. The criteria of selection, other than method of

estimation, are that the estimation be on 1 sample of milk from 1 woman, and that the milk be mature. The former basis of selection was made to avoid the narrowing of the apparent distribution by the inclusion of mean values and of values from large pooled samples of milk which are, from this point of view, mean values. Early milk was excluded because of the atypical N levels which it is known to show. An arbitrary dividing line at 14 days was taken between early and mature milk.

Most of the estimations used give results in terms of N × 6·37, and to avoid unnecessary arithmetic all results were reduced to that basis. The constant recommended for conversion of total N to protein is now 6·38 (*A.O.A.C. Handbook*, 4th ed., 1935 ; Hawk *et al.*, 1947).

The histogram shown, Fig. 6, is based on a total of 915 estimations. The assembly from which the histogram was constructed was neither purposive nor random, but could probably best be described as haphazard. The stage of lactation at which the milk samples were taken varied from 3 weeks to 2 years ; the size of the samples varied from part of a single nursing to the mixed yield of 2 whole days. The distribution shown is therefore a composite of every source of variation to which milk N is subject.

Variation between Breasts

The investigations examining this variation are few. Brown *et al.* (1922) gave a total of 6 pairs of analyses from 3 women ; a difference in the N content between the breasts was found, but it was not statistically significant. Denis and Talbot (1919), Elsdon (1928), Myers (1927) and Schlossmann (1900) also made observations but none of them found a significant difference.

These investigations all examined the difference between right and left in general. It seems possible, however, that consistent differences in N content may exist between the two breasts of any one woman although the right need not bear the same relation to the left in another woman. Thus the figures given by Brown *et al.* (1932) are, in g. protein per 100 ml. :

	Subject 6			*Subject 7*		*Subject 8*	*Mean*
L.	1·089	1·116	0·920	0·968	1·162	1·169	1·071
R.	1·083	1·104	0·938	0·981	1·166	1·181	1·076

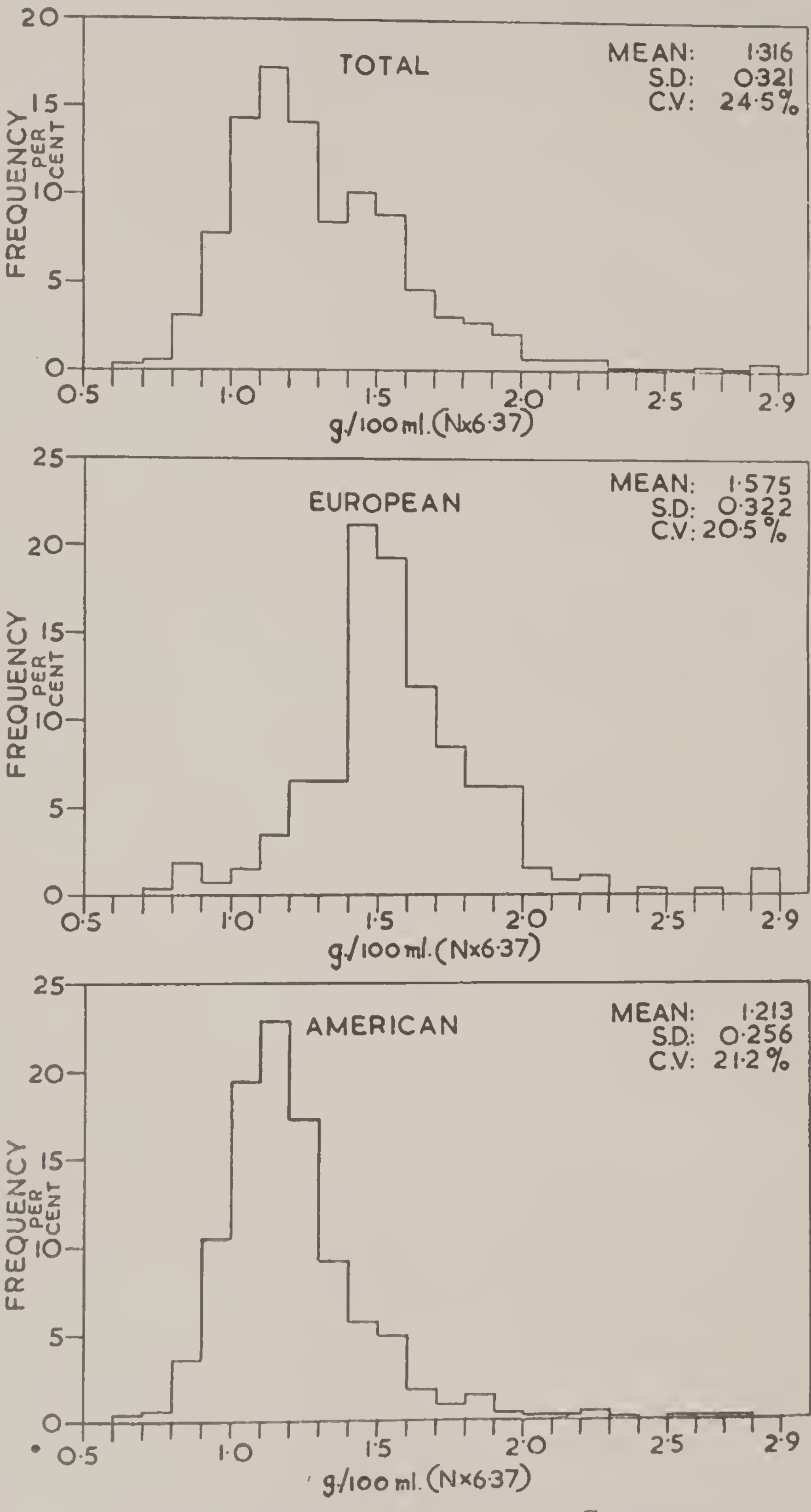


FIG. 6 DISTRIBUTION OF PROTEIN CONTENT

These show a mean difference of 0·005 between right and left breasts. But the 3 women, taken separately, show mean differences between breasts of 0, 0·009 and 0·012. It is obvious that before any conclusion can be reached as regards difference between breasts, several analyses of milk taken simultaneously from each breast of individual women must be made. None of the work done records a sufficient number of observations from the same woman.

Variation during a Nursing

Macy *et al.* (1931) examined the composition of the milk from the entire first and last halves of the nursing period. Analysis of the results which they obtained showed a highly significant difference in N content, the N from the last half being 3 per cent. higher than that in the first. The observations of Denis and Talbot (1919) on the composition of the milk taken before and after nursing show the after milk to be 2·75 per cent. higher in N than the fore milk, but the difference is not statistically significant. The fore and after milks were also compared by Carter and Richmond (1898), who estimated ostensibly " true " protein, but no significant difference was found. Vincent and Vial (1933*d*) record protein as being at a maximum about the middle of a nursing. In more recent work on the same problem, Waisman and Petazze (1947) examined the milk from the beginning, middle and end of each of 16 nursings from 10 women. Again there was no significant systematic change during nursing, although the tendency was to increase, the mean difference between first and last milk being 4·4 per cent. Lowenfeld *et al.* (1927) made a similar study of colostrum. Again the after milk was found to be about 3 per cent. higher in N than the fore milk but the difference was not significant.

Although the mean differences between first and last milk are small, the individual differences found can be quite large. The greatest difference recorded by Waisman and Petazze was from 146 to 177 mg. N per 100 ml. milk, an increase of 21 per cent. Also, although the means increase from first to last milk, this does not always occur when individual nursings are considered.

The reality of a systematic change in total N from beginning to end of a nursing thus remains in doubt, but the magnitude of the change, if there be one, is such that as far as the nutrition of the infant is concerned the change is of negligible importance compared with the other variations in protein intake to which the infant is subject. If N content and volume are independent, then assuming a loss of 15 ml. at the end of a nursing capable of yielding 150 ml., and assuming the N content of the milk to rise to 177 mg. per 100 ml. as recorded by Waisman and Petazze, the total N lost by the infant would be 26·5 mg., that is, 10·9 per cent. of the potential amount obtainable; but of that 10·9 per cent., 9 is accounted for purely by the volume of milk lost, leaving about 1·9 accountable to the change in N content of the milk from beginning to end of the nursing.

The standard deviation of individuals about the mean N content is 162·7 $\pm$ 14·4 mg., of stages of nursing about the mean 162·7 $\pm$ 6·4 mg., and of volume about mean nursing volume 150 $\pm$ 93 ml.; this last was calculated from all available nursing volumes for the early morning nursing, a total of 52. A fixed nursing period was taken to eliminate diurnal variation. The average N yield is 245 mg. N. The variation produced about this mean by differences due to stage of nursing is 9·6 mg., to individual variation 21·6 mg., and to variations in volume 151·5 mg. Expressed as percentages, variations in the N content of the milk will, on the average, alter the amount consumed by the infant by 4 to 8 per cent., and average volume variations will alter it by 60 per cent.

Diurnal Variation

This variation in total N or protein content has been examined by Denis and Talbot (1919), Macy *et al.* (1931), and Deem (1931), on 6, 3 and 7 subjects, respectively. None of these results shows a definite systematic variation, though the differences between successive nursings by the same subject can be substantial. These are plotted against time of day in Fig. 1. Nims *et al.* (1932*a*) give their results in the form of maxima and minima at different times of the day; maxima occurred most often during the afternoon and minima during the night.

Denis *et al.* (1919) examined the N.P.N. levels throughout the day but found no appreciable variation.

Again the changes produced in the intake of N by the infant by this variation are very small.

Macy *et al.* (1931) also examined the diurnal variation in casein N, albumin N, N.P.N. and amino-acid N, but in none of these was it appreciable.

Variation with Stage of Lactation

There appears to be general agreement in textbooks and in reviews of the composition of human milk that total N of human milk decreases throughout lactation. Many of the reviews given as preliminaries to original work have either claimed this change explicitly or implied it in the values quoted from previous workers. There is considerable apparent evidence for this, the decrease being rapid for the first few days *post partum* and a slow decline continuing thereafter.

There is no doubt of the high initial level of the total N immediately after parturition, and of the fall for the first 10 days (Lowenfeld *et al.*, 1927 ; Widdows *et al.*, 1935). These workers showed that the N falls in the first 2 or 3 days from about 8 g. protein per 100 ml., range 5·6 to 11·5 g., on the 1st day to about 3 g. on the 4th day, remaining then more or less constant but with the suggestion of a decline to 14 days.

Widdows *et al.* also give figures for the protein content of the pre-natal secretion from 8 days before labour. The protein levels found were high but of the same magnitude as that in the 1st day *post partum.* On the day of labour, however, the level rose to 12·6 g. per 100 ml. The data on protein content from this paper are given in Table 3, from which it can also be seen that the range of variation tends to decrease as the milk moves towards maturity. The post-natal data are plotted in Fig. 7.

After the colostrum period has passed, and 10 days should see the major changes from colostral to milk characteristics completed, there is a period, called by Holt *et al.* (1915) " transitional ", during which some workers have claimed a continued decline, though at a much lower rate. This period is taken by Holt, who gives detailed data, to extend to 30 days. Over that period a

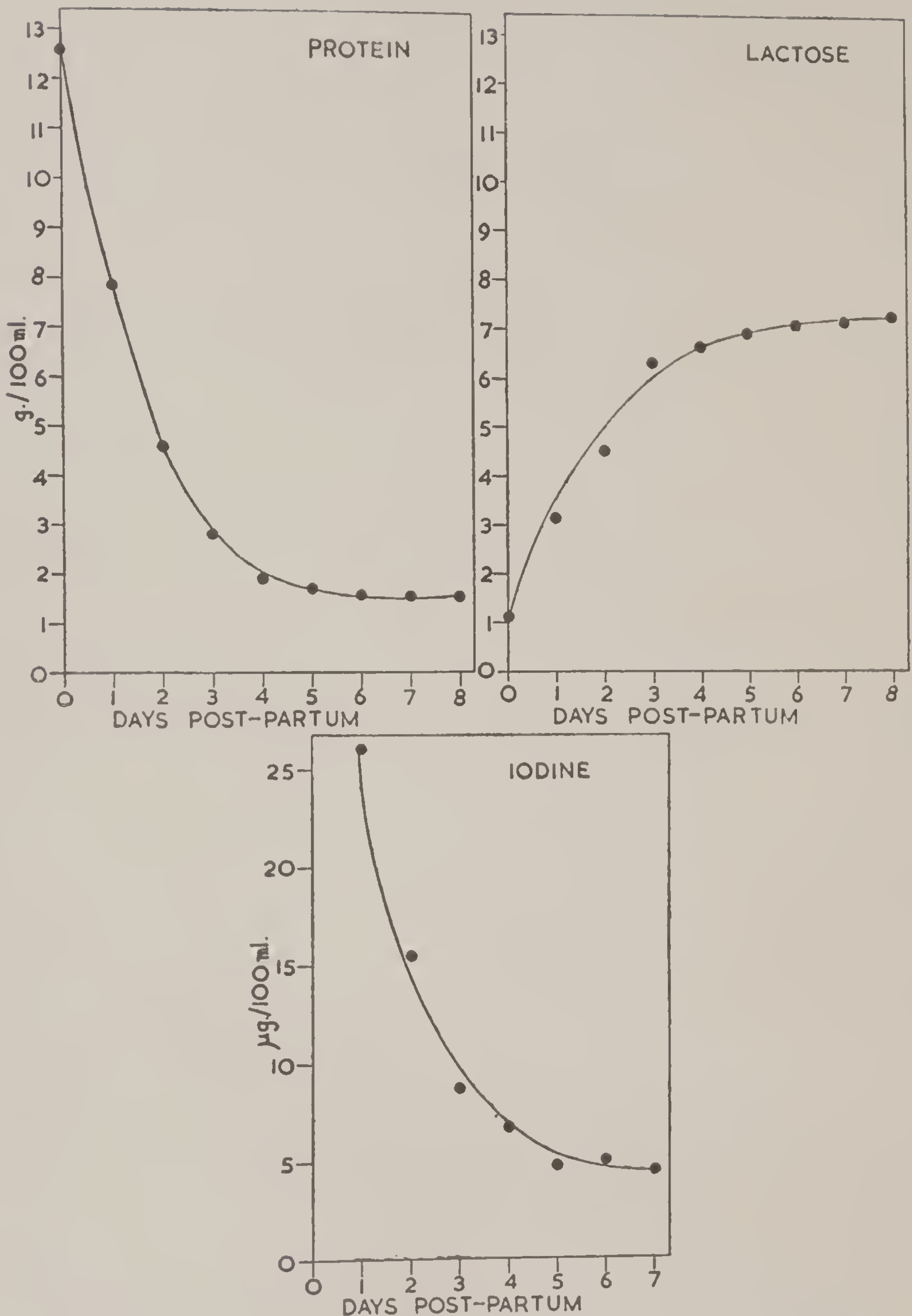


FIG. 7 PROTEIN, LACTOSE AND IODINE CONTENTS OF COLOSTRUM
(Protein and lactose, Widdows *et al.*, 1935; I, Elmer and Rychlik, 1934.)

decline appears to occur, to judge from summary tables which are, in general, all that have been published. In general, also, it is claimed that the N content continues to decline slowly throughout lactation. The results of Pfeiffer (1894), Camerer and Söldner (1896*b*), Adriance and Adriance (1897), Carter and Richmond (1898), Schlossmann (1900 ; 1902), Holt *et al.* (1915), Abderhalden (1923), Bell (1928), Widdows *et al.* (1930), and Kon and Mawson (1950) are shown in Table 4. These results cannot be combined in one summary set of values owing to the different methods of estimation used and lack of information, in some instances, about the number of analyses included in a given average value.

TABLE 3

PROTEIN CONTENT OF PRE-NATAL SECRETION AND OF COLOSTRUM (Widdows *et al.*, 1935)

	Weeks before Birth					Day before Labour	In Labour	Days after Birth							
	8,7,6,5	4-3	3-2	2-1	1-0			1	2	3	4	5	6	7	8
Mean protein g./100 ml.	8·40	9·28	9·18	7·54	8·16	8·39	12·60	7·86	4·60	2·80	1·90	1·70	1·57	1·55	1·55
No. of observations	15	8	7	8	16	11	4	11	17	27	25	20	20	13	23
Range as percentage of mean	110	140	112	95	117	181	59	146	154	254	128	63	33	53	70

This table shows how weak the evidence is for a decline in N content throughout lactation. Of the older records the two which show most obvious evidence of decline are those of Carter and Richmond and Bell, but these effectively record only the decline up to the end of the 1st month. Camerer and Söldner record data after the 1st month, but the extent of the decline is not impressive, especially with such small numbers. The most convincing evidence available of such a decline is that of Kon and Mawson.

Gardner and Fox (1925) combined the results of a number of investigations and their table is reproduced in Table 5. The values for protein which they deduced certainly indicate a decline, but the only measure of dispersion they give is the maximum and

TABLE 4

VARIATION OF PROTEIN CONTENT WITH STAGE OF LACTATION

(No. of samples in brackets)

Source / Time	Pfeiffer (1894) Protein g.%	Adriance and Adriance (1897a) Protein g.%	Schlossmann (1900; 1902)[1] Protein g.%	Camerer and Söldner (1896) Protein g./100 ml.	Carter and Richmond (1898) Protein g.%	Abderhalden (1923) Protein g./100 ml.	Holt *et al.* (1915) Protein g./100 ml.	Bell (1928) Protein g.%	Widdows *et al.* (1930) Protein g./100 ml.	Escudero and Pierangeli (1940-41a) Protein g./100 ml.	Kon and Mawson (1950) Protein g./100 ml.
0-7 days	—	—	—	3·11 (5)	2·22 (9)	—	2·52 (2)	2·00 (88)	2·673 (82)[2]	—	—
7-14 „	—	—	1·81	1·76 (7)	2·06 (15)	2·61	1·06 (1)	1·73 (88)	1·449 (26)[2]	—	1·72 ± 0·07 (12)
14-21 „	—	1·55	—	—	1·61 (13)	2.01	1·58 (2)	—	—	1·078 (5)	1·54 ± 0·03 (15)
21-30 „	2·98	1·63	1·94	1·37 (5)	—	1·85	—	1·37 (35)	1·175(2)[2]	—	1·47 ± 0·04 (35)
1-2 months	2·04	1·54	1·60	1·31 (3)	1·43 (8) (> 30 dys.)	1·70	1·17 (1)	1·30 (32) 1·21 (14)	1·174 (16)	1·188 (16)	1·36 ± 0·01 (141)
-3 „	1·99	1·49	1·35	1·04 (3)	—	1·57	1·05 (2)	—	1·111 (19)	1·128 (29)	1·25 ± 0·03 (126)
-4 „	1·77	1·67	1·25	1·04 (4)	—	1·55	1·14 (6)	—	—	1·091 (29)	1·22 ± 0·01 (143)
-5 „	1·45	1·31		—	—	1·47	1·14 (2)	—	0·986 (7)	1·175 (21)	1·18 ± 0·02 (79)
-6 „	1·54	1·43	1·29	—	—	—	1·10 (1)	—	—	1·112 (33)	1·18 ± 0·03 (35)
-7 „	1·53	1·20		0·91 (5) (> 6 mths.)	—	—	1·15 (2)	—	—	1·122 (46)	1·01 ± 0·04 (10)
-8 „	1·69	1·08	1·31 (> 7 mths.)	—	—	—	1·28 (3)	—	—	1·118 (35)	—
-9 „	1·54	0·86	—	—	—	—		—	0·934 (12)	1·153 (36)	—
-10 „	1·71	0·86	—	—	—	—		—	—	0·154 (30)	—
-11 „	1·47	0·64	—	—	—	—	1·00 (3)	—	—	1·093 (22)	—
-12 „	1·73	1·18	—	—	—	—	—	—	—	1·120 (23)	—
-13 „	1·66	1·02	—	—	—	—	—	—	—	1·170 (19)	—
-14 „	—	0·65	—	—	—	—	1·18 (3) >(13 mths.)	—	—	1·225 (16)	—
-15 „	—	0·64	—	—	—	—	—	—	—	1·144 (14)	—

[1] Quoted from Czerny and Keller (1923).
[2] Approximate time periods.

TABLE 5

VARIATION OF COMPOSITION WITH STAGE OF LACTATION
(Gardner and Fox, 1925)

Secretion	No. of Samples	Protein g./100 ml.			Carbohydrate g./100 ml.			Ash g./100 ml.			Fat g./100 ml.		
		Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
Colostrum 1-6 days	179	0·66	2·394	4·86	3·00	7·032	11·90	0·13	0·2707	0·48	0·56	3·082	6·54
Colostrum 7-12 days	100	0·95	1·894	2·85	3·80	6·790	9·20	0·10	0·2365	0·38	1·54	3·428	7·65
Transitional milk 13-20 days	220	0·56	1·726	2·88	4·26	6·961	10·70	0·11	0·2155	0·34	1·00	3·944	9·91
Mature milk 1-9 months	583	0·37	1·390	3·59	4·26	7·003	10·41	0·09	0·1914	0·37	1·00	3·902	8·81
Late milk 9th month	72	0·09	1·073	2·31	5·64	7·039	8·36	0·13	0·1759	0·23	0·07	4·061	9·46

minimum values and, as they point out, the average apparent variations with stage of lactation are well within the limits of individual variation.

Earlier work by Woodward (1897) shows a tendency towards some change in the protein content of colostrum, but it is much less marked. This estimation was based on 6 women.

TABLE 6

PROTEIN CONTENT OF COLOSTRUM

(Woodward, 1897)

Days of lactation	1	2	3	4	5	6	7
Protein, g. per cent.	2·1	1·9	1·9	1·8	1·8	1·7	1·8
No. of observations	6	3	6	3	4	1	2

The highest 1st day protein level was 2·68 and only 2 women showed a definite decline of protein with time after parturition, one even showing a constant increase over the 7 days.

Woodward associated high colostral protein with the presence of colostral corpuscles. It is unlikely that the corpuscles are present in sufficient quantity to account for the increased protein, but the observation suggests that high protein is a true characteristic of colostrum.

Castellanos and Lizarralde (1943), using mixed samples of colostrum, found no change in N content in the first few days of lactation but the mean values they found, 2·06, 2·19, 2·21 and 2·13 g. protein per 100 ml. for the 2nd to the 5th day inclusive, were higher than that which they commonly found for mature milk.

Sikes (1906*b*), in a paper dealing with a method of estimation, gives 2·5 g. protein on the 4th day, 1·9 on the 8th, 1·76 on the 9th and a single value of 2·27 on the 11th day.

Birk (1910) estimated total N from the 2nd to the 8th day on 2 women, and made estimations successively throughout the course of the day. Both subjects showed a definite decline in N content, but it is interesting to note that the subject who showed the higher initial level and the greater rate of decline showed a much lower yield than the other and the milk retained colostral characteristics (yellow colour) up to the 8th day. The milk with the low initial

N level had reached a steady N content by the 3rd day. This is a finding similar to that of Widdows and Lowenfeld (1933) who found that high initial protein contents occurred with low initial yields.

Waller *et al.* (1941) found that in 27 women N content rose to the 2nd or 3rd day *post partum* and thereafter declined to about the 7th day. Again the scatter appears to have been considerable, but as only means, maxima and minima are given these data cannot be examined further, and it must be noted that one or two subjects showing atypical secretion could considerably modify the mean values.

Bell (1928) found that the protein content of the milk of 88 women decreased from the 5th to the 9th day *post partum* from an average of 2·00 to 1·73 g. (total N $\times$ 6·37). Values for one individual woman show a decrease from 2·20 g. at 5 days to 1·32 g. at 42 days.

Hammett (1917) found the total N content of the milk of 8 women to follow an irregularly declining course to the 11th day. The milk was sampled at the same hour each day.

TABLE 7

Total Nitrogen Content of Colostrum

(Hammett, 1917)

Days *post partum*	3	5	7	9	11
N, mg./100 g.	552 $\pm$ 45	273 $\pm$ 45	253 $\pm$ 45	264 $\pm$ 45	230 $\pm$ 45
Number of subjects	8	8	8	8	7

Again the scatter was very great, but the difference between days is significant ($P < 0{\cdot}001$). The difference between subjects was not, in this case, very pronounced ($0{\cdot}2 > P > 0{\cdot}1$), but removal of the variation due to subject difference reduces the standard error to $\pm$ 37.

Meigs and Marsh (1913-14) found the N content of milk up to the 9th day *post partum* to be 249 mg. per 100 g. compared with 178 mg. for mature milk. The 5 early samples of milk were composites ; the 2 mature were individual.

Camerer and Söldner (1896*a*) found a drop in N content from 957 mg. per 100 ml. at 26 to 51 hours to 524 mg. at 56 to 61 hours and 242 mg. at 9 days. Thereafter the decline was irregular.

The observations of Nims *et al.* (1932*b*) on 3 women for 5-day periods throughout lactation give the following values for N content of the milk (Table 8). This shows a significantly higher

TABLE 8

VARIATION OF NITROGEN CONTENT WITH STAGE OF LACTATION

(Nims *et al.*, 1932*b*)

Time	6th week mg./100 ml.		6th month mg./100 ml.		8th month mg./100 ml.		14th month mg./100 ml.	
Subject	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
1 . .	191	14·7	158	7·5	160	6·8	155	6·1
2 . .	201	9·4	164	15·9	161	2·6	159	8·7
3 . .	222	3·1	189	11·6	190	3·0	186	3·8
Mean .	205	15·9	169	18·2	170	15·0	165	18·3

N content in the 6th week than for the later samples, but no difference between the later samples themselves. There is no way of telling whether the level tails off slowly from the 6th week to the 6th month or whether the level at the 6th week is part of a relatively rapid decline which flattens out fairly soon thereafter.

Rearrangement of the data of Escudero and Pierangeli (1940-41*a*) according to stage of lactation gives the following table:

TABLE 9

PROTEIN CONTENT AT DIFFERENT STAGES OF LACTATION

(Escudero and Pierangeli, 1940-41*a*)

Months	0·5-2·0 [1]		2-6		6-10		>10
No. of observations .	21		112		147		127
Average protein (N × 6·37), g./100 ml.	1·162		1·1225		1·135		1·178
Mean difference .		0·0395		0·0125		0·043	
S.E. of difference .		0·06		0·0289		0·0265	

[1] Average 39 days.

Obviously none of these differences is significant. A scatter diagram of the same data (not reproduced) shows even more strikingly the lack of any definite trend of protein content with stage of lactation as found by this work.

Freudenberg (1941) describes the case of a woman in whom lactation had persisted for 4 years. The total dry matter rose to over 50 per cent. and the protein to 5·5 per cent. The protein,

however, contained no casein. This protein level as a percentage of total dry matter does not differ much from that of mature milk.

By and large the body of evidence is for a considerable decline from parturition to about 10 days *post partum*, the average decline following a course something like that depicted by Widdows *et al.* (1935). Thereafter the question of a decline is much more doubtful. It seems likely that the colostral decline in total N may continue well into the 2nd month, though at a much slower rate than in the first 10 days. For the period beyond the 2nd month there appears to be doubtful evidence of a continued slow decline.

McCance and Widdowson (1951) report for 3 samples of " regression " milk a relatively high protein level, 2·4, 1·9 and 9·4 g. per 100 ml. This they found to correspond with high acid and alkaline phosphatase levels comparable with those in colostrum.

In all the individual lactation records which have been examined and in all those which have been quoted here, it is apparent that, as with volume, the N content follows an individual course and that the differences in N content of the mature milk between individuals are as great as those from any other cause (*cf.* Table 8 just quoted from Nims *et al.*, 1932*b*). This is especially evident in Table 1 from Deem's (1931) subjects. It may be that the decline in N throughout lactation observed by some authors is due to the presence among their subjects of a few women whose milk shows atypical behaviour. The decline in colostrum is considerably obscured by " atypical " secretions, such as those of Woodward's (1897) subject who showed an increase in N over the colostral period. Even in the colostral period it must be assumed that perhaps 20 per cent. of women will show a secretion which is " atypical " in this respect at least.

The individual woman follows an individual pattern of milk production, and a few individuals deviating markedly from the average trend may, in investigations involving small numbers, appreciably distort the apparent average trend.

Variation with Parity

Although a fair number of analyses have been made of milk from mothers whose parity was recorded, there are very few

analyses of milks taken at the same stage of lactation from the same women in different lactations ; that is, such data as there are on parity are cross-sectional instead of what is required to test this possible variation, namely, longitudinal data. Nims *et al.* (1932*b*) give the total N for 2 women after 56 weeks' lactation in two successive lactations, and in both the total N of the earlier lactation was about 10 per cent. above that of the later.

Data giving total N and the parity of the mother for the 1st, 2nd, 3rd and 4th parities have been combined from Nims *et al.* (1932*b*), Brown *et al.* (1932), Holt *et al.* (1915), and Denis and Talbot (1919), and the computed averages are given in Table 10. These figures appear to corroborate the deduction suggested by the successive studies of Nims *et al.*, but it must be remembered that here there are several variables operating simultaneously, notably, age of the infant (*i.e.*, stage of lactation) and age of the mother. Also it is to be expected that parity will vary with the age of the mother, and in fact, the mean age of these groups does increase with parity. To get an unequivocal estimate of the effect of parity from such cross-sectional data would require a much greater number of observations.

TABLE 10

VARIATION OF COMPOSITION WITH PARITY

(Calculated from Holt *et al.* (1915), Denis and Talbot (1919), Brown *et al.* (1932), and Nims *et al.* (1932) : number of subjects in brackets)

Milk Component \ Parity	1	2	3	4
Nitrogen mg./100 ml.	(21) 229 ± 10	(22) 202 ± 11	(13) 191 ± 9	(9) 165 ± 6
Fat g./100 ml.	(9) 3·17 ± 0·32	(16) 4·10 ± 0·36	(9) 3·82 ± 0·27	(9) 4·07 ± 0·32
Lactose g./100 ml.	(9) 7·53 ± 0·26	(16) 7·42 ± 0·17	(9) 7·27 ± 0·14	(9) 7·30 ± 0·14

Bell (1928), in a paper on the composition of the milk of 88 women about whom no information is given, claimed that no

correlation between the age or parity of the mother and the composition of the milk could be found.

Lowenfeld *et al.* (1927) and Widdows *et al.* (1935) found that the protein content of colostrum from primiparae is, for the first 2 or 3 days *post partum*, higher than that of the colostrum from multiparae. The data were presented in graphical form. Numerical values derived from the graph in the latter paper give mean values for the first 2 days of 6·49 g. protein per 100 ml. for primiparae and 4·88 for multiparae, but the scatter was great and the difference is not statistically significant. The values for total nitrogen recorded by Waller *et al.* (1941) for 5 (1st day) and later 20 primiparae and 2 (1st day) and later 7 multiparae show no consistent difference.

Variation with Age

Since parity will tend to be correlated with age, any variation in the composition of milk which is associated with one of these will show at least a reflected association with the other unless they have opposite effects. The data for parity in Table 10 when re-arranged according to age of the mother (Table 11) indicate a slight association of total N content with age.

TABLE 11

VARIATION OF COMPOSITION WITH AGE

(Calculated from Holt *et al.* (1915), Denis and Talbot (1919), Brown *et al.* (1932), and Nims *et al.* (1932) : number of subjects in brackets)

Milk Component / Age in Years	6-21	22-25	26-31	>32
Nitrogen mg./100 ml.	(11) 238 ± 23	(18) 210 ± 14	(22) 187 ± 7	(14) 186 ± 8
Fat g./100 ml.	—	(11) 3·41 ± 0·32	(19) 4·29 ± 0·27	(12) 3·59 ± 0·32
Lactose g./100 ml.	—	(11) 7·55 ± 0·12	(19) 7·40 ± 0·14	(12) 7·35 ± 0·19

The largest single recent study of milk composition is that of Escudero and Pierangeli (1940-41*a*), in which it seems safe to assume that the methods of estimation and collection of the milk were uniform. In this work analyses were made of over 400 samples (see footnote, p. 1) of milk from 50 women at different stages of lactation. The data for " total protein " (total N × 6·37) have been classified according to age of mother in Table 12. The differences between age groups just reach the 0·05 *P* significance level, but in any case show no consistent trend.

TABLE 12

VARIATION OF COMPOSITION WITH AGE

(Calculated from Escudero and Pierangeli (1940-41*a*) : number of samples in brackets)

Milk Component \ Age in Years	16-21	22-25	26-31	>32
Protein g./100 ml.	(71) 1·108 ± 0·031	(113) 1·175 ± 0·023	(123) 1·119 ± 0·016	(100) 1·175 ± 0·023
Fat g./100 ml.	(72) 3·92 ± 0·169	(122) 4·59 ± 0·133	(122) 4·17 ± 0·125	(102) 4·56 ± 0·184
Lactose g./100 ml.	(72) 7·360 ± 0·056	(122) 7·224 ± 0·054	(122) 7·480 ± 0·051	(102) 7·367 ± 0·061

Effect of Diet

Deem (1931) examined the effect of diet on the protein content of the milk, Table 2. There is a significant difference between diets, but it is completely overshadowed by the differences between the individual subjects. The tendency was for the high protein diets to produce a high protein milk, but while, for volume, the institution diet was the poorest, for protein content of the milk it was comparable with the two high protein diets. The maximum protein difference observed, between the high protein and the home or low protein diets, would produce a difference in available

protein to the child of about 14 per cent. As with volume, however, the danger of carry-over effects must be recognised in interpreting this work.

Hoobler (1917) attempted to assess the effect of types and quantities of protein in the diet on milk production. The percentage N of the milk does not appear to have been affected in any regular way by the diets given.

Average results obtained by Escudero and Pierangeli (1940-41*b*) for the protein content of the milk of the women attending their " ginegaladosia " in 1938, before the introduction of dietary supervision, and in 1939-40 after its introduction, showed a rise in the protein content of the milk from 1·06 g. per 100 ml. to 1·14 g. per 100 ml. After 1939 the diet of the women was supervised and, if necessary, supplemented. The same authors in 1943 published data for the mean composition of colostrum from two groups of women ; one group (101) received a hospital diet during and after pregnancy, the other group (126) received the maternity institute diet. The mean protein content of the milks from the two groups was 1·89 and 2·18 g. per 100 ml. In view of the variation which occurs in colostrum, interpretation is difficult.

Table 13, computed from the data of Escudero and Pierangeli (1940-41*a*), shows the effect of variation of each measured component of the diet on the protein, fat and lactose of the milk. Here a significant effect of protein content of the diet on protein content of the milk can be seen.

Daggs (1940) found that in all subjects (4) examined milk secretion was stimulated by daily ingestion of 5 g. cystine. Analysis of milk from one subject indicated that fat rose and lactose fell during the treatment but the change was small.

Ružičić (1934) found evidence to show that diet had a slight but not statistically significant influence on protein content.

Certain foreign proteins appear to find their way into the milk ; for instance, according to Shannon (1921), egg-white protein may give rise to allergy to egg-white in the infant. Very large amounts of egg-white appear to be necessary before the protein appears in the milk. Stuart (1923) did not find egg-white protein in milk and doubts the importance of Shannon's observations.

TABLE 13

EFFECT ON DIET ON COMPOSITION
(Calculated from Escudero and Pierangeli (1940-41*a*) : number of samples in brackets)

Milk Component \ Level in Diet g. per kg. body-weight	Protein		
	1·0-1·5	1·5-2·0	2·0-2·5
Protein g./100 ml.	(48) 1·095 ±0·031	(276) 1·143 ±0·015	(92) 1·259 ±0·033
Fat g./100 ml.	(48) 3·55 ±0·18	(276) 4·19 ±0·085	(91) 5·11 ±0·18
Lactose g./100 ml.	(47) 7·42 ±0·076	(279) 7·39 ±0·033	(94) 7·18 ±0·081

Milk Component \ Level in Diet g. per kg. body-weight	Fat			
	1-1·5	1·5-2·0	2·0-2·5	>2·5
Protein g./100 ml.	(59) 1·151 ±0·024	(168) 1·143 ±0·021	(169) 1·167 ±0·020	(22) 1·342 ±0·080
Fat g./100 ml.	(57) 3·26 ±0·19	(167) 3·88 ±0·10	(171) 4·96 ±0·10	(21) 6·06 ±0·29
Lactose g./100 ml.	(59) 7·40 ±0·074	(166) 7·38 ±0·058	(174) 7·30 ±0·046	(22) 7·38 ±0·076

Milk Component \ Level in Diet g. per kg. body-weight	Carbohydrate		
	5-6	6-7	7-8
Protein g./100 ml.	(121) 1·168 ±0·024	(215) 1·146 ±0·017	(66) 1·177 ±0·032
Fat g./100 ml.	(123) 4·09 ±0·13	(214) 4·40 ±0·11	(67) 4·67 ±0·19
Lactose g./100 ml.	(121) 7·30 ±0·052	(217) 7·38 ±0·042	(66) 7·38 ±0·061

Hammett (1917) has suggested that there is a differcnce in protein content of milk between European and American women. The mean protein level found from investigations on European women, particularly Germans, tends to be higher than that found in American investigations. Talenti (1933) for 6 samples of milk of Italian women records protein between 2·2 and 3·0 per cent. It is impossible to tell whether this is due to different diet and environment or to a racial difference. Since nearly all the European work is considerably older than the American, it seems possible that changes in methods of estimation may be largely responsible for the apparent difference. The distribution differences are shown in Fig. 6, giving means of 1·575 and 1·213 g. per 100 ml. An overall mean found by Kon and Mawson (1950) of 1·26 (S.D. 0·19) is close to the American figure.

Nitrogen Partition

The quantitative separation of the nitrogenous components of human milk has been a matter of considerable difficulty as it is not easy to precipitate the protein fractions, casein, albumin and globulin, by the use of salts and acids. The conditions necessary for the precipitation of human milk casein are much more difficult to establish than those for the casein of cow's milk. Wang and Wood (1930) found that cow's milk casein was easily precipitated by hydrochloric acid, acetic acid or lactic acid alone or by rennin alone, but that the casein of human milk was not precipitated by any of these alone. The optimum *p*H of precipitation of human milk casein was 4·97. Fuld and Wohlgemuth (1907), Engel (1908*a*, *b*), Bauer and Engel (1911), Demuth (1924), Meyer (1926), and Trendtel (1927) all studied the conditions for the precipitation of the several N fractions in human milk and the conditions for its curdling, but they give no values for the different fractions.

The recent technique of electrophoresis by which the homogeneity of a protein can be examined should be of value in establishing more clearly the optimum precipitation conditions. This has already been done by Mellander (1947) for human milk casein and by Biserte and Masse (1948) for ante-natal secretion, 7th month, and colostrum protein. The latter workers found much the same picture for both, with 5 fractions, of which the

two of lowest mobility were lactalbumin and lactoglobulin, the lactalbumin being the most abundant.

Derrien *et al.* (1950), from solubility estimations, claimed the presence in human milk of at least 15 different proteins, of which 4 are related to caseinogen.

The difficulties of quantitative estimation of the N partition lie in the precipitation methods used. It is a simple matter to precipitate all the protein, more difficult to ensure that only protein has been precipitated ; even if it becomes possible to prepare fairly " pure " samples of the separate proteins it is very much more difficult to achieve that separation quantitatively.

Camerer and Söldner (1896*b*) precipitated the total protein by an acetic and tannic acid mixture (Almen's reagent), and by estimating the whole milk N and the N of the filtrate deduced the protein N. It was shown by them that the N.P.N. made up from 10·4 to 21·8 per cent. of the total N, with a mean of 17 per cent. Woodward (1897) also made a check for N.P.N. which he found to be about 10 per cent. of the total N (protein precipitated by tannin), but, on the whole, the early workers either estimated total N or precipitated all the protein and measured it either by N estimation or by weighing without any reference to N.P.N. substances.

Czerny and Keller (1923) quote Lehmann (1894) as giving values for casein and albumin separately (casein 1·2 and albumin 0·5 per cent.). König (1903) quotes individual data from Decaisne, Doyère, Pfeiffer and Zaliski which, taken together, give mean values of 0·80 and 1·02 per cent. for casein and albumin, with ranges of 0·18 to 1·90 and 0·29 to 2·35 per cent., respectively. No mention is made of N.P.N., which is probably included in the albumin fraction. Without knowing the exact methods used by these authors in making the separation it is not strictly justifiable to combine the results, but the values do give some indication of the order of the results found by these early workers. Out of 173 analyses recorded by König, of which many are means of a greater original number of individual samples, only the 20 above and 3 sets of mean values give separate estimates of casein and " albumin ".

Holt *et al.* (1915) attempted to separate the proteins by precipitating casein with acetic acid and assuming the remainder to be albumin. They obtained mean values, on 24 samples, of 182 mg. total N, 64 mg. casein N and 118 mg. albumin N. They recognised that the " albumin " fraction would contain N.P.N. Meigs and Marsh (1913-14) made a protein separation on two samples of milk, precipitating what they referred to as casein and globulin with magnesium sulphate, and albumin with acetic acid and heat ; one of these samples was colostrum and the other early milk. Casein + globulin was found to be 53·1 per cent., albumin 17·3 and N.P.N. 29·6 per cent. of the total N for colostrum and 63·0, 12·2 and 24·8 per cent., respectively, for early milk. Friedheim (1909) found on 8 samples the total N to be 187 mg., the whey N of acid-treated milk 85 mg. and the whey N of rennin-treated milk 106 mg. per 100 ml.

Macy *et al.* (1931) and Nims *et al.* (1932*b*) separated the proteins by the A.O.A.C. method, *i.e.*, by precipitation of the casein by acetic acid at 40° C. with subsequent precipitation of the albumin after neutralisation with NaOH at steam bath heat. In the former work N.P.N. was also estimated after precipitation of all the protein by tungstic acid. Here the average total N (3 women, a total of 32 estimations) was 165 mg. per 100 ml., casein N forming 31·6, albumin 38·3 and N.P.N. 20·1 per cent. of that. Casein, albumin and N.P.N. together accounted for 90 per cent. of the directly estimated total N.

In a later paper of this series Erickson *et al.* (1933) investigated the methods of estimation of the nitrogenous constituents. The casein was precipitated at *p*H 4·6 with acetic acid and acetate, and the combined albumin and globulin fraction was subsequently precipitated with trichloroacetic acid. This method gave a total N content of about 160 mg. per 100 ml., with casein, albumin + globulin and N.P.N. making up 38, 38 and 23 per cent. of this. Two methods used for precipitating total protein gave consistently different results for the residual N.P.N. When the protein of freshly drawn milk was precipitated by tungstic acid the N.P.N. was always less (initially 10 per cent. less) than when the protein was precipitated by trichloroacetic acid. The difference increased with time of standing from 0 to 72 hours before precipitation.

The total N.P.N. by either method also increased with time of standing, but it is not clear from which of the protein fractions the increase derives. The values given indicate that it may come from the albumin + globulin fraction. The authors attribute the difference in N.P.N. between the two methods to peptone and proteose N which is precipitated by tungstic acid but not by trichloroacetic acid. The fact that this peptone N was low in milk analysed immediately after extraction and increased with time indicated the presence of a proteolytic enzyme, and as after 72 hours the N.P.N. found after tungstic acid precipitation also showed an increase, it appears that proteolysis down to some products lower than peptones had occurred. A fairly high initial urea content with no ammonia, and the change with time to no urea with almost quantitative increase in ammonia indicate the presence of a urease also. Free amino-acid N also increased with time of standing.

Erickson *et al.* (1934) examined the detailed partition of the N.P.N. more closely and in relation to the blood levels of total N and its components at the time of milk extraction. The blood and milk levels of total N.P.N., urea, free amino-acid N and uric acid N were of comparable magnitude, but there did not appear to be any close relation between them.

TABLE 14

NON-PROTEIN NITROGEN PARTITION IN MILK AND BLOOD

(Erickson *et al.*, 1934)

Component / Source	N.P.N.	Urea N	Amino-acid N	Uric acid N
Milk, mg. per 100 ml.	38·2 ± 11	15·8 ± 2·9	5·7	2·2
Blood, mg. per 100 ml.	40·8 ± 11	17·9 ± 5·6	7·7	3·8

These values include three for colostrum, which had a high N.P.N. content, including high uric acid and creatine. One subject whose milk had been tested at intervals throughout lactation became febrile at 80 days *post partum* and the milk reverted, in its N.P.N. content, to colostral character ; this change was believed to be a reflection of the blood picture. The fractions

of the N.P.N. which showed increase in fever were urea and amino-acid N. There was some slight indication that the maximum N.P.N. values occur at night and the minimum during the late morning.

Denis *et al.* (1919) estimated the N.P.N. and urea in milk and blood, 20 samples from 20 women, and for both the milk and blood levels were significantly correlated ; $r_{NPN} = + 0{\cdot}626$ and $r_{urea} = + 0{\cdot}702$ ($P < 0{\cdot}01$ for both coefficients). The regression lines and equations are shown in Fig. 8. There was no apparent diurnal variation in N.P.N.

Waller *et al.* (1941) studied prenatal and postnatal N.P.N. and protein values. Casein was precipitated by acetic acid and acetate, and globulin from the filtrate by magnesium sulphate at *p*H 6·8 to 7·2. These workers give only mean values, and even for early milk the N.P.N. seems exceptionally high. It will be seen that the casein : globulin ratio moves from less than 1 to greater than 1 as the milk moves through the colostral period. In this scheme the albumin is probably included, at least in part, in the N.P.N. The final globulin figure appears too low to represent the combined non-casein protein (cf. p. 39 and Table 16).

TABLE 15

NON-PROTEIN NITROGEN, CASEIN AND GLOBULIN CONTENTS OF COLOSTRUM

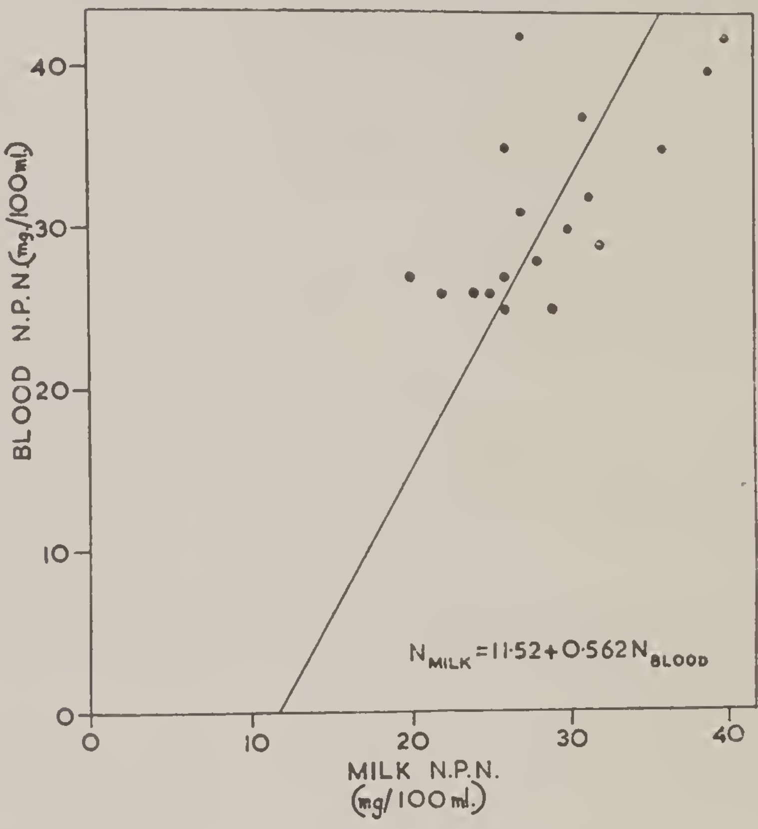
(Waller *et al.*, 1941)

Time	N.P.N. No.	N.P.N. mg./100 ml.	Casein N No.	Casein N mg./100 ml.	Globulin N No.	Globulin N mg./100 ml.
Prenatal . . .	15	105	43	334	41	825
1 to 4 days *post partum*	7	91	40	323	39	556
5 to 14 days *post partum*	21	84	84	144	83	78

All the data available on N partition are gathered in a summary table (Table 16). In constructing this table certain assumptions have been made about what fraction of the N is precipitated by the reagents used. It has been assumed that treatment of the milk in the cold or at temperatures up to 40° C. with acetic acid (A.O.A.C. casein method I), HCl and lactic acid (Van Slyke and Baker, 1918) or acetic acid and acetate buffer, precipitate caseinogen and only caseinogen. All the caseinogen is precipitated

only at the iso-electric point pH 4·6, but it has been assumed that where not specifically stated, the acidity has been near enough to this point to produce nearly complete precipitation.

After removal of the caseinogen it is assumed that acetic acid and acetate, or neutralisation of the original acetic acid with



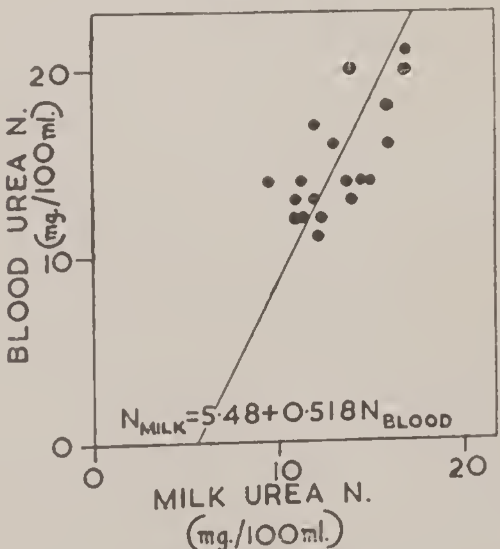


FIG. 8 N.P N. AND UREA IN BLOOD AND MILK
(Denis *et al.*, 1919.)

TABLE 16.—NITROGEN PARTITION (mg./100 ml. milk)

Source	Total N	Casein N	Albumin N	Globulin N	N.P.N.	Peptone N	Amino-acid N	Urea N	Uric Acid N	Creatinine, preformed	Total	Description of samples
Camerer and Söldner (1896*b*, 1898)	283 (10)	—	—	—	43 (10)	—	—	—	—	—	—	Colostrum
	180 (34)	—	—	—	33 (34)	—	—	—	—	—	—	Milk
Woodward (1897)	158 (1)	—	—	—	44 (1)	—	—	—	—	—	—	Colostrum
Friedheim (1909)	187 (8)	85 (8)	< . . . . 81 (8) . . . . >			—	—	—	—	—	—	Milk
Grosser (1913)	178 (4)	—	—	—	49 (4)	—	—	—	—	—	—	Milk
Meigs and Marsh (1913-14)	257 (2)	+Globulin 167 (2)	39 (2)	—	71 (2)	—	—	—	—	—	—	Average of colostrum and early milk. Time not stated
Holt *et al.* (1915)	179 (24)	63 (24)	< . . . . 116 (24) . . . . >			—	—	—	—	—	—	Milk
Denis and Minot (1919)	—	—	—	—	33 (3)	—	7·2 (3)	12·6 (3)	2·5 (3)	1·5 (3)	5·0 (3)	Milk
Denis *et al.* (1919)	—	—	—	—	28 (71)	—	6·0 (38)	11·9 (71)	2·7 (10)	1·2 (42)	3·4 (38)	Milk
Macy *et al.* (1931)	165 (32)	52 (32)	<. . . 63 (32). . . >		33 (32)	—	4·3 (32)	—	—	—	—	Milk
Nims *et al.* (1932*b*)	175 (10)	68 (10)	54 (10)	<. . . 53 (10) . . . >		—	—	—	—	—	—	Milk
Erickson *et al.* (1933)	—	60 (*c*)	< . . . 62 (*c*) . . . >		39 (*c*)	3·3 (*c*)	5·3 (*c*)	10·1 (*c*)	—	1·6 (10)	3·5 (10)	Milk
Erickson *et al.* (1934)	—	—	—	—	38 (14)	—	5·7 (15)	15·8 (10)	2·2 (9)	1·7 (2)	5·8 (2)	Partly colostrum, partly milk
Scott (1934)	195 (3)	< . . . 142 (3) . . . including peptone N . . . >			53 (3)	—	—	—	—	—	—	Milk
Plimmer and Lowndes (1937)	192 (31)	46 (31)	<. . . 99 (31) . . . >		47 (31)	—	—	—	—	—	—	Milk
Beach *et al.* (1941*b*)	162 (4)	49 (4)	< . . . 77 (4) . . . >		36 (4)	—	—	—	—	—	—	Milk
Waller *et al.* (1941)	1152 (81)[1]	323 (40)[2]	—	556 (39)[2]	91 (7)[2]	—	—	—	—	—	—	Colostrum [1] 2-4 days [2] 1-4 days
	345 (135)[3]	144 (84)[4]	—	78 (83)[4]	84 (21)[4]	—	—	—	—	—	—	[3] 5-9 days [4] 5-14 days
Waisman and Petazze (1945)	173 (27)	70 (27)	<. . . 72 (27) . . . >		31 (27)	—	2·9 (27)	14·8 (27)	—	—	—	Milk
Waisman and Petazze (1947)	163 (48)	65 (48)	<. . . 64 (48) . . . >		33 (48)	—	—	15·2 (30)	—	—	—	Milk
Escudero and Rothman (1949)	174 (47)	—	—	—	32 (47)	—	—	—	—	—	—	Milk

In brackets number of samples from the same or a less number of subjects. (*c*) is believed to be a composite sample.

NaOH followed by further addition of acetic acid (A.O.A.C. albumin method I) and heating on a steam bath will precipitate albumin and globulin. Saturation with magnesium sulphate precipitates caseinogen and globulin, or, if caseinogen be already removed, only globulin.

Trichloroacetic acid is assumed to precipitate all milk proteins together or severally according as individual proteins have been removed by other means or not, so that treatment with trichloroacetic acid will leave only N.P.N., peptones and proteose bodies in solution. Tungstic acid and zinc hydroxide are each assumed to precipitate all proteins along with peptones and proteoses.

Data derived from enzymic clotting of caseinogen are not included, since the product thrown out of solution is not caseinogen and does not appear to be quantitatively comparable with the product of acid and salt precipitation.

Tannic acid is assumed to throw out all proteins and an indefinite amount of N.P.N.

Amino-acid Composition of Human Milk Proteins

Amino-acids of proteins are estimated by chemical and microbiological methods. The relative merits of these methods have been reviewed by Block and Bolling (1945), Block and Mitchell (1946-47), and Schweigert and Snell (1946-47).

Before the work of Plimmer and Lowndes (1937) only isolated values for one or two amino-acids were available for human milk (Fürth and Nobel, 1920 ; Tillmans and Alt, 1925 ; Tillmans *et al.*, 1928). Thereafter several analyses are available in terms of the essential amino-acids. These are listed in Tables 17 and 18, which give the amino-acids as g. per 16 g. N and as mg. per 100 ml. milk. For comparison the values for cow's milk are given also.

In general the levels found by different groups of workers correspond fairly closely. Some of the more pronounced differences, for example, between the mean values found by Block and Bolling (1946) and Macy (1949), are possibly due, at least in part, to differences in method of estimation ; it is probable that the microbiological methods used by Macy are not yet adequately correlated with chemical methods. But where both Block and Bolling and Macy used microbiological methods, for leucine,

*iso*leucine and valine, the mean values found still show appreciable, though not statistically significant, differences.

Block and Bolling point out that there is great individual variation in relative concentrations of amino-acids and that many of the differences are statistically significant. These authors also found a significantly lower level of cystine in the milk of coloured than of white mothers. As some of the analyses quoted in Tables 17 and 18 were for milk samples from individual subjects, some for pooled milk from white and coloured subjects and some for unspecified pooled milk, the two sources of variation, between individuals and between races, will probably go some way towards explaining the apparent discrepancies.

Assuming approximate correspondence between the microbiological and chemical methods of estimation of amino-acids, it would appear that the differences in proportions of those present in milk protein are not of much importance. The view that the higher concentration of cystine in human than in cow's milk protein made the protein of human milk nutritionally superior has recently received damaging criticism, for although cystine appears to be essential for many lower mammals it was not found to be essential for the growth or nitrogen balance of the infant (Albanese *et al.*, 1949).

In an attempt to find some other property of human milk which would justify claims that it is superior as a source of protein for infants, Block and Bolling (1950) examined the amino-acid distribution in the N.P.N. fraction. These results also are presented in Table 18 and show no appreciable difference, but, of the total N.P.N. of breast milk, 80 per cent. is amino-N, compared with 50 per cent. in cow's milk.

The higher overall levels of all the amino-acids in colostrum (Table 18) is explained by the higher protein content, but the change in the proportions of amino-acids present (Table 17) is probably due to the different proportions of casein and lactalbumin in colostrum (Block and Bolling, 1946).

Vecchio and Cutillo (1950) found that the N : S ratio rose from 8·66 at the first day *post partum* to 11·81 in established milk. The absolute amounts of N and S both fell during the period but the latter fell more steeply.

TABLE 17

AMINO-ACID CONTENT OF HUMAN AND OF COW'S MILK PROTEIN
(Calculated as g. per 16 g. N)

Source / Amino-acid	Human Milk										Cow's Milk								
	Mature (1)	Mature (2)	Mature (3)	Mature† (4)	White Mature (5)	Coloured Mature (5)	Colostrum (5)	Mature (6)*	Early Colostrum (6)*	Late Colostrum (6)*	Casein (2)	Lact-albumin (2)	N.P.N. Mature (7)	N.P.N. Colostrum (7)	Total Protein (1)	Casein (1)	Lact-albumin (1)	β-Lacto-globulin (1)	N.P.N. (7)
Arginine	4·3	5·1	5·1	4·8	3·7	3·7	5·5	4·2±0·1	3·3	4·0±0·2	4·2	5·5	—	—	4·3	4·2	3·9	3·0	—
Histidine	2·8	1·8	1·6	1·8	2·8	2·7	2·6	2·3±0·1	1·8	2·5±0·1	1·7	1·8	—	—	2·6	3·0	2·1	1·5	—
Lysine	7·2	6·8	6·3	6·7	6·7	6·7	6·4	6·7±0·2	5·2	7·2±0·2	5·9	7·3	5·3	6·2	7·5	7·9	9·6	11·4	4·9*
Tyrosine	5·2	5·2	6·3	5·2	5·4±0·1	5·4±0·3	5·4±0·2	—	—	—	5·9	4·8	2·7	5·1	5·3	6·9	4·4	4·3	1·3
Tryptophan	1·9	2·2	2·4	2·2	1·7±0·1	1·5±0·1	2·0±0·2	1·8±0·1	1·4	1·8±0·03	1·0	2·7	0·4	0·8	1·6	1·2	2·5	2·0	0·6
Phenylalanine	5·6	—	—	5·4	5·5±0·3	5·8±0·3	5·9±0·3	3·9±0·1	3·1	4·0±0·1	—	—	—	—	5·7	5·6	5·4	5·2	—
Cystine	3·4	3·5	2·5	2·9	2·1±0·1	1·4±0·1	2·5±0·1	—	—	—	0·7	4·8	1·1	1·4	1·0	0·3	4·1	3·5	1·1
Methionine	2·2	2·0	2·3	2·0	2·4±0·1	2·3±0·2	1·8±0·1	1·1±0·5	1·1	1·5±0·05	3·1	1·5	2·3	—	3·4	3·5	2·7	3·6	1·8
Threonine	4·6	—	—	4·5	4·5±0·1	4·5±0·1	5·0±0·1	5·0±0·2	3·8	5·0±0·1	—	—	3·1	4·4	4·5	4·1	5·4	6·0	1·9
Leucine	9·8	—	—	16·2	10·2±1·1*	10·1±1·0*	7·9±0·8*	9·3±0·3	7·4	9·7±0·3	—	—	4·9	4·8	11·3	9·9	10·4	15·3	4·0*
*iso*Leucine	7·5	—	—	5·3	7·6±0·8*	7·2±0·7*	5·4±0·6*	5·9±0·2	4·5	6·2±0·1	—	—	4·6	3·2	8·5	6·5	6·4	7·0	3·1*
Valine	8·8	—	—	4·7	9·9±1·0*	9·0±1·0*	6·9±0·7*	7·0±0·3	5·2	6·7±0·2	—	—	5·1	4·8	8·4	6·7	6·4	5·5	3·9*
Glycine	—	—	—	0	—	—	—	—	—	—	—	—	—	—	2·3	2·1	—	—	—

(1) Block and Mitchell (1946-47). (2) Plimmer and Lowndes (1937). (3) Beach *et al.* (1941*a*). (4) Williamson (1944).
(5) Block and Bolling (1946). (6) Macy (1949). (7) Block and Bolling (1950).
* Microbiological methods. † Taken in this form from Block and Bolling (1946). It implies a protein N of 115 mg. per 100 ml.

TABLE 18

PROTEIN AMINO-ACID CONTENT OF HUMAN AND OF COW'S MILK
(Calculated as mg. per 100 ml. Milk)

Source / Amino-acid	Human Milk															Cow's Milk				
	(*a*) Mature (1)	Mature (2)	Mature (3)	Mature (4)	(*a*) White Mature (5)	(*a*) Coloured Mature (5)	(*b*) Colostrum (5)	Mature (6)*	Early Colostrum (6)*	Late Colostrum (6)*	Casein (2)	Lact-albumin (2)	N.P.N. Mature (7)	N.P.N. Colostrum (7)	(*c*) Total Protein (1)	(*d*) Casein (1)	(*e*) Lact-albumin (1)	(*f*) β-Lacto-globulin (1)	N.P.N. (7)
Arginine	43	46	40	67	37	37	86	43	74	63	12	34	—	—	128	92	11	3	—
Histidine	28	16	12	25	28	27	41	24	41	38	5	11	—	—	77	66	6	1	—
Lysine	72	62	50	94	67	67	100	70	118	113	17	45	10	21	223	173	27	10	10*
Tyrosine	52	47	50	73	54±1	54±3	84±3	—	—	—	17	30	5	17	157	151	12	4	3
Tryptophan	19	20	19	31	17±1	15±1	31±3	19	32	28	3	17	1	3	48	26	7	2	1
Phenylalanine	56	—	—	77	55±3	58±3	92±5	40	70	62	—	—	—	—	169	123	15	5	—
Cystine	34	32	20	41	21±1	14±1	39±2	—	—	—	2	30	2	5	30	7	12	3	2
Methionine	22	18	18	29	24±1	23±2	28±2	12	25	24	9	9	5	—	101	77	8	3	4
Threonine	46	—	—	63	45±1	45±1	78±2	52	85	78	—	—	6	15	134	90	15	5	4
Leucine	98	—	—	228	102±11*	101±10*	123±13*	97	166	151	—	—	9	16	336	217	29	13	9*
*iso*Leucine	75	—	—	75	76±8*	72±7*	84±9*	61	101	97	—	—	9	11	252	142	18	6	7*
Valine	88	—	—	66	99±10*	90±10*	108±11*	73	117	105	—	—	10	16	249	147	18	5	8*
Glycine	—	—	—	0	—	—	—	—	—	—	—	—	—	—	68	46	—	—	—

(1) Block and Mitchell (1946-47).
(2) Plimmer and Lowndes (1937).
(3) Beach *et al.* (1941*b*).
(4) Williamson (1944).
(5) Block and Bolling (1946).
(6) Macy (1949).
(7) Block and Bolling (1950).

* Microbiological methods.
(*a*) Converted to this form assuming protein N 160 mg./100 ml.
(*b*) Converted to this form assuming protein N 250 mg./100 ml.
(*c*) Converted to this form assuming protein N 475 mg./100 ml.
(*d*) Converted to this form assuming casein N 350 mg./100 ml.
(*e*) Converted to this form assuming lactalbumin N 45 mg./100 ml. (Rowland, 1938-39).
(*f*) Converted to this form assuming β-lactoglobulin N 14 mg./100 ml. (Rowland, 1938-39).

Biological Value of Human Milk Proteins

Kon and Mawson (1950) reported the biological values and true digestibilities of the proteins of human and cow's milk for rats. The mean biological values found were 85·7 ± 3·55 and 84·7 ± 3·24, and the true digestibilities 79·2 ± 2·46 and 92·5 ± 1·04 per cent. The low true digestibility of human milk was associated with the production of intestinal disturbances.

Other work with rats in which rate of weight increase was the criterion, *e.g.*, that of Haam and Beard (1935) and Das and Guha (1936), gives conflicting results not interpretable in terms of milk components. The value of such work is doubtful, and comparisons of biological values in terms of rate of weight increase of infants (*e.g.*, Faber and Sutton, 1930 ; Wardlaw and Dart, 1932) are complicated by differences in energy intake and possibly by differences in vitamin content. Ujsághy (1940) found that retention of N and S was greater in the breast fed than in the artificially fed infant.

Breast milk is thought to contain toxic products at times, *e.g.*, during menstruation. The evidence is trifling ; *cf.* Eltz (1932), who was unable to grow lupin seeds in milk from a menstruating subject.

Fat and Lipoid Matter

It does not seem profitable to assemble all the data on the fat content of human milk as has been done for protein. There was considerable doubt whether significant variation occurred in N content over a day, but there can be no reasonable doubt that the fat content of milk does vary with time of day and method of sampling. The magnitude of such variation is also so great as to be physiologically significant and cannot be ignored.

In Fig. 9 such estimates as are available from full-day samples of milk have been gathered together and give a mean of 3·33 g. per 100 ml. with a standard deviation of 0·57 g. (C.V. 17·8 per cent.). This is based on only 299 observations from all sorts of subjects.

Diurnal Variation

That this variation occurs and is very considerable has been shown by many workers ; some of the mean results found are

shown in Fig. 1, p. 4. The most striking cyclical variation is shown by the data of Denis and Talbot (1919) and of Deem (1931). Most investigators recorded the variation for 1 day in several women ; Vincent and Vial (1932) studied the variation in the same women over successive days and found that it continued more or less regularly during the whole period covered.

There is great individual scatter in fat content. Taking all the individual values together, the mean difference between

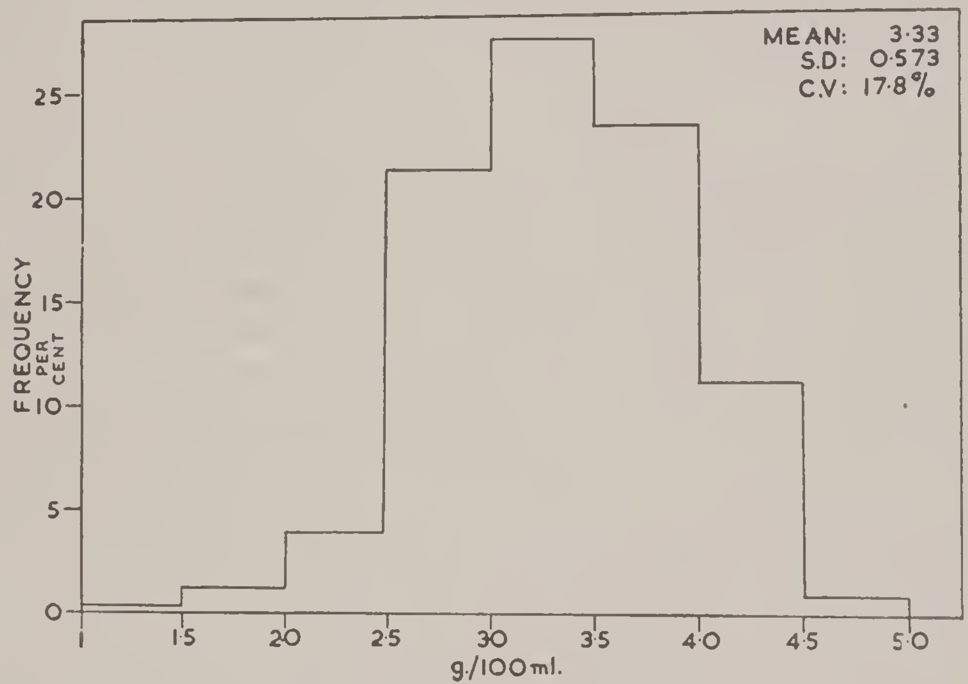


FIG. 9 DISTRIBUTION OF TOTAL LIPID CONTENT

highest and lowest is 2·52 g. per 100 ml., which is about 75 per cent. of the general mean fat content.

Yet this general trend is not apparent in all studies and there is no agreement about its causes. Macy *et al.* (1931) estimated the fat content of the milk of 2 subjects at 4-hour intervals throughout a day and did not find a clear cyclical variation but only a tendency for the fat to decrease towards morning.

More recent work by Gunther and Stanier (1949) has shown that the diurnal variation in fat content occurs even when the milk is fully extracted after injection of pitocin, but Shaw (1942) found that in cows treated with this hormone diurnal variation in the fat content of milk disappeared. Gunther and Stanier claimed also from their results that the variation is a true rhythm and appears

to be unaffected by feeding or by periods of rest or activity. This is in contradiction to Ružičić (1936), who claimed that there was no physiological rhythm but only a reflection of meal-time rhythm.

In general these investigations show that, if a typical value is to be obtained, the fat estimations must be made on the whole day's milk. Collection of samples from different subjects at the same time of the day will not give a satisfactory comparison, since an appreciable number of subjects deviate considerably from the " type " form of variation shown by the mean curve.

Variation during a Nursing

This is another major source of variation, commonly known, and well established by several investigators (Denis and Talbot, 1919 ; Telfer, 1924 ; Lowenfeld *et al.*, 1927 ; Macy *et al.*, 1931 ; Lucignani, 1934 ; Waller, 1943 ; Salmi, 1944-45 ; and Sydow, 1944-45). The results of all these workers show that the after milk is higher in fat than the fore milk, and some workers, taking the milk in 3 parts, fore, middle and after, found a consistent increase in the fat content from beginning to end of the nursing.

It has been suggested, particularly by Lowenfeld *et al.* and Sydow, that the magnitude of this increase in fat content depends on the method of extraction of the milk. Fat concentration increases with force of expression of the milk. Lowenfeld *et al.* found that when the milk was expressed manually at first and by breast pump later, the fat increased from beginning to end much more than when the methods of extraction were reversed. Sydow found that the milk obtained by milking dry was richest in fat, and that appearing by spontaneous discharge was poorest, $3{\cdot}56 \pm 0{\cdot}16$ and $2{\cdot}89 \pm 0{\cdot}05$ per cent., respectively, but the numbers of donors were only 5 and 13.

Lucignani (1934) investigated the fat content of the milk yielded in consecutive minutes from a series of nursings and found that it rose to a maximum at about 10 min. and thereafter declined slightly to 15 min. ; the pattern was consistent although the difference between maximum and minimum values varied greatly.

Some older authors found no increase in the fat content of the milk from beginning to end of a nursing, or even a reverse change,

i.e., the early milk richer in fat than the later. Gölz (1940) explains such results, and his own which show only a slight increase, by attributing the increase from fore to after milk to difference in viscosity between fat and solids-not-fat, the effect of which becomes apparent with inexpert evacuation of the breasts. When the breast is expertly evacuated, with manipulation, the milk, he says, will be uniform throughout in fat content. This may be true, but it leaves unresolved the problem of the physiological importance to the infant of incomplete extraction, or variable force of extraction by it, when suckling. If, moreover, the change in fat content were entirely due to variable force of extraction acting in a purely mechanical way it would be expected that, with consistently low extraction force, the milk of successive nursings would increase in fat content until a limiting value was attained, or that the nursing following a weak extraction nursing would at least show a high initial fat content. No such result has been shown. It may be, on the other hand, that the fat content of the milk is a direct reflection of the rate of fat secretion and that force of extraction has a reflex action on secretion rate.

It is a reflex of this sort that Vincent and Vial (1933*b*) believe to operate rather than a change in size of fat globule from beginning to end of nursing. If, as has been claimed, large fat globules in the ductules of the glands at the beginning of a nursing (*i.e.*, after rest) impede the progress of the smaller and so reduce the amount of fat in the fore milk, they should also impede the flow of milk, and so volume in successive minutes of a nursing should parallel fat content, which it does not appear to do. Nor is it easy to explain the end decline in fat observed by Lucignani on this basis. Empirically, however, large fat globules do appear to be associated with high fat content, and small globules with low fat content, a result consistent with expectation from dimensions, since the volume of a globule varies as the cube of its diameter. But large globules have been associated with low protein and small with high protein. Bentivoglio (1933) found for milks with predominantly large, medium or small globules the ratios $10 \times$ protein : fat to be $1{\cdot}75 \pm 0{\cdot}14$, $2{\cdot}398 \pm 0{\cdot}13$ and $4{\cdot}62 \pm 0{\cdot}34$, respectively, the effect being produced both by the fall of fat and by the rise of protein content with decrease in the globule size.

This suggests that the difference lies in the secretion of the acini and not in mechanical blockage of the ductules. It is possible that both effects operate, but it might be profitable to give more serious consideration in future studies to the possible reflex alteration of composition.

The reflex hypothesis is supported by the work of Waller (1943), who found that the increase in fat from fore to after milk was usually about twice as great in milk from the breast suckled first as in that from the second, and that the difference occurred whatever the order of suckling.

Failure to follow this pattern of fat production is most noticeable in the first few days of lactation, and Lowenfeld *et al.* (1927) claimed that it was confined to primiparae, but of the 20 pairs of observations which they give, only 4 refer to multiparae and so the claim cannot be accepted without further showing. Of Denis and Talbot's (1919) 40 subjects, 5 showed a reversed pattern and 1 no change ; of these 3 were secundiparae and 3 primiparae, and 5 of the 6 were yielding mature milk.

Although it seems likely that the change in fat content over a nursing would be of physiological significance to the infant, there is no definite evidence to that effect. Sydow (1944-45) found differing average weight increases per month of groups of infants fed on milk obtained at different stages of a nursing.

(*a*) Extracted before nursing : 866 ± 42 g.
(*b*) Extracted after nursing : 765 ± 62 g.
(*c*) Spontaneously discharged milk : 636 ± 97 g.

These differences are not significant and are, in any case, not consistent, but the low rate of increase shown by the group receiving spontaneously discharged milk is suggestive.

Variation with Stage of Lactation

No investigation has indicated any consistent trend of fat content with stage of lactation in mature milk. In most studies, if such a trend had existed, it might have been masked by the use of part-day or part-nursing samples, but examination of values for fat based on whole-day samples has not shown any such trend.

There are indications that fat content increases to about the end of the first month. Gölz (1940) found an increase from 2·8

to 4·1 per cent. of fat from the 3rd to the 30th day of lactation, and Fox and Gardner (1924), from the combined results of three investigations, record a mean fat content of 2·8 g. per 100 ml. for days 1 to 12 and of 3·6 g. for days 12 to 30. These investigations were based on reasonably large numbers of samples, although the individual results are not given, but neither appears to have been based on whole-day samples. The finding of Fox and Gardner might indeed be referable to differences in technique in the different investigations, since nearly all the results for early milk (1 to 12 days) were these authors' own work, while the values for later milk came from Denis and Minot (1918) and from Wacker and Beck (1921). Castellanos and Lizarralde (1943) found no consistent trend in the fat content of mixed samples of the first 5 days' milk. Comparison of the fat contents of milk from 5 to 21 days with that of milk from 1 to 9 months, as recorded by Holt *et al.* (1915) for whole-day samples, shows no significant difference, but the early milk samples were only 4 in number. Gardner and Fox (1925) also show a rise (Table 5, p. 27) in fat content, but this becomes poorly defined once mature milk has been established.

At the present stage of chemical work on the fat content of colostrum no definite conclusion can be drawn, but the additional evidence of the increase in number of stainable fat globules with development of the milk, and the association of this increase with the decrease in colostral corpuscles (which appear finally to degenerate to fatty material), does suggest that if an investigation using adequate numbers of whole-day samples was made an increase might be found.

Variation with Volume

Some of the older workers stated that there was an inverse relation between fat content and daily milk volume. Of more recent work Salmi's (1944-45) analyses of surplus milk given to a milk bank show no variation with volume, although he claimed that they indicated a direct relation between fat and volume; Gölz claims a direct relation, finding the difference not between the fat and volume of milk of individual days from individual women, but in the average fat content of the milk of

" hypergalactic " and " hypogalactic " women. He gives the following results :

	Per cent. of Cases	Mean Fat per cent.
Strongly hypergalactic (greater than 800 g. daily at the 7th day)	33·5	3·45
Slightly hypergalactic	46·5	3·50
Normal	12·0	2·90
Hypogalactic (less than 200 g. daily at the 7th day)	8·0	2·80

Gölz does not give individual results from which the significance of these differences may be computed, but if they are significant it seems that the contrast is between " hypergalactic " women and others and that there is no continuous variation with milk yield. It seems odd to refer to 80 per cent. of the cases studied as hypergalactic and 12 as " normal ", and it seems probable that Gölz's concept of the normal may be a bit low.

Kon and Mawson (1950) found no systematic variation of fat with volume, but claimed that sudden fluctuations in volume secreted by an individual woman may produce changes in fat content which may then vary inversely with the yield.

The diurnal variation in volume already discussed (pp. 3-5) combined with the diurnal variation in fat content produces another type of variation of fat content with volume. The results of most investigations on these diurnal variations indicate that the fat content and volume throughout the day are in anti-phase, but the work of Vincent and Vial (1932, 1933*a*, *b*), covering consecutive days on the same subjects, shows the fat and volume to vary in phase. Although this is one finding against many, such consecutive data are more convincing than cross-sectional data, but again further work must be awaited before the position is clarified.

A scatter diagram of the relation between fat and volume for whole-day samples was prepared in which all the reliable data were assembled ; many of them are mean values. This diagram shows no indication of any trend of fat with volume, and it does not appear worth while either to reproduce it or to compute a regression or correlation coefficient.

It has been suggested that fat varies with volume in one of three ways : that there is (*a*) a difference between the fat contents

of milks which are given in consistently high or low yield, which difference appears possible but has not been established ; (*b*) a continuous variation of fat content with milk yield, independent of consistently low or high yield, for the existence of which there is no good evidence ; and (*c*) a diurnal relation, which almost certainly exists, but whether it is direct or inverse probably varies with the subject.

Variation with Age and Parity

By reason of the great importance of sampling for the estimation of fat content, acceptable data with corresponding information on age and parity are very few. Those for mature milk (Holt *et al.*, 1915 ; Denis and Talbot, 1919 ; Brown *et al.*, 1932 ; and Nims *et al.*, 1932) have been assembled and the means and S.E.'s are shown in Tables 10 and 11, pp. 32, 33.

Of the data derived from part-day samples, or where the sampling procedure is not detailed, the most reliable are probably those given by Escudero and Pierangeli (1940-41*a*). There the numbers are large and although the samples were of mixed "surplus" milk not accurately defined (see footnote, p. 1), the composition does not differ from that of other part-day samples. The data, classified according to age of mother (parity is not given) are in Table 12, p. 34. Salmi (1944-45) gives means for the different age groups of his subjects, the fat content rising from 3·8 below 25 years to 4·4 per cent. above 35 years, but there is no way of telling whether this change is significant. Flori (1934) also indicates a rise in the fat content with age and parity, but the changes are not significant.

Day-to-day Variation

This is very considerable. Salmi (1944-45) gives the fat content on 14 successive days for 6 subjects ; the ranges of variation for the individual subjects over that period were : 1·4 to 4·2 ; 2·5 to 4·1 ; 1·6 to 3·8 ; 2·3 to 3·9 ; 1·8 to 4·2 ; 2·3 to 4·1 g. per 100 ml.

There is some indication in this work that individual mothers follow individual patterns in fat content, but analysis of the data showed no significant difference between individuals. Deem's

(1931) data show a very highly significant difference between individuals (Table 1, p. 14) but she gives no day-to-day data ; all of her values are means for a week.

This great day-to-day variation, although slightly offset by the difficultly discernible individual variation, makes it possible that even a whole-day sample may not be adequate to decide the normality or otherwise of the fat content of the milk. It is possible that this variation may be due to irregular extraction of the milk.

Seasonal Variation

Salmi (1944-45) claims a seasonal variation in fat content, minimum values occurring during the spring and maximum in the autumn. For January to June the mean value found was 3·9 and for July to December 4·5 per cent. Only means are given for each month, without indication of the numbers of samples ; and there is no gradual transition from spring to autumn values, all the values for the second semester, excepting December, being higher than those for the first semester. The difference between spring and autumn is given for 8 individual subjects ; the fat content of the milk from 6 of these was higher in autumn than in spring, and from 2 lower. The mean rise, from 2·87 to 3·45 g. per cent., is not significant.

Effect of Diet

Deem (1931) estimated the fat content of the milk of 5 women each on 7 different diets (Table 2). The differences between individual women were significant and so also was the difference between diets ($P < 0{\cdot}001$). As with the dietary effect on the protein content, caution is needed in the interpretation of these results, for the diets were given to each subject in consecutive weeks without interval and no account was taken of possible carry-over effects. The highest fat contents were found on the high-fat and low-protein diets, but the low-protein followed immediately on the high-fat and the high-fat on the high-sugar diet, so that, although a dietary effect appears to have operated, the apparent correspondence of the change in fat content with the change in diet may be spurious. The lowest fat was on the high-protein

diet which immediately followed the institution diet, and the fat content on the home diet immediately following the high-protein was the second lowest, so it seems possible that the low fat yield with the high-protein diet is a real effect.

Polonovski (1933) showed that addition of 100 g. glucose daily to the diet tended to increase the fat content of the milk within one week. When the daily supplement of glucose was increased to 200 g. the milk fat increased by 0·5 to 1·0 g. per 100 ml. (10 to 30 per cent.) within a week, and the results further indicated that the increase continued progressively, at least up to 15 days. This work emphasises that the apparent dietary causes of fat increase found by Deem may be confused by carry-over effects.

Ružičić (1934) found that the fat content of the milk rose to a maximum when a butter and meat diet was given, and fell to a minimum when a diet of meat and bread or of bread alone was given. During starvation milk yield fell and fat content rose. This finding was not confirmed by Gunther and Stanier (1949).

The same author, Ružičić (1938), although finding no definite general relation between fat content and yield, found a tendency for women giving an exceptionally high yield to have a milk low in fat. It is possible that this finding may be related to diet.

The only other major attempt to assess the effect of diet on milk composition is that of Escudero and Pierangeli (1940-41*a*). Here neither the sampling of the milk nor the method of dietary survey was completely satisfactory (see footnote, p. 1), but the increases in fat content of the milk with increase in fat content of the diet which they found are very striking (Table 13).

Apart from these direct studies, most of the recorded work on the effect of diet on milk composition was done to compare fat during periods of relative plenty and of restriction. This is not a very satisfactory method at best, but it is particularly unsatisfactory for fat, which we have seen may be considerably affected by carbohydrate intake. The relative values of diets are customarily assessed qualitatively on their protein content, and most investigations on the effect of restricted diet on milk fat are based on such a qualitative assessment of diet. German workers after the 1914-18 war found no difference in the percentage of milk fat from that estimated pre-war. Lavagne and Mathieu (1943)

5

during the period of food shortage in France in 1942 also found no change in fat, but they compared their results with a textbook value for fat content which may have been low anyway, about 3 per cent. Also, these workers estimated the fat in early milk, 5th, 8th and 11th days, and the sampling was not completely satisfactory, and indeed the same may be said of sampling in all these investigations on the effect of war-time restrictions.

Salmi (1944-45) found in milk from a milk bank in Helsingfors a reduction in fat content from 4·1 per cent. in 1939 to 3·1 per cent. in 1942, a fall of 25 per cent. This fall was claimed by Sydow (1944-45) to be probably, at least in part, due to changes in methods of extraction of the milk. Salmi gives no individual values from which the significance of his means may be estimated.

Of greater interest is Sydow's report on the milk of 10 Polish mothers released from Ravensbrück. Milk was taken for analysis 11 to 15 days after the arrival of these women in Gothenburg and again 2 months later ; there was no significant change or even the suggestion of a change in fat content. It seems possible that the lactational response to dietary improvement after severe restriction may be very rapid and so that the time between release of Sydow's Polish subjects and the extraction of the first milk samples was sufficient to allow the milk secreted to regain normal composition. This is certainly suggested by Polonovski's (1933) work on glucose supplements added to a normal diet.

Some of the investigations on the effects of pre-war and war-time diets may be confused, to some extent, with a possible racial difference in fat content. Some such racial difference is suggested by the general mass of data ; for example, Ylppö (1928) found 4·52 per cent. fat for Finnish, and Gölz (1940) 3·5 per cent. for German women. There is, however, no way of reliably establishing this difference with the data available, and it is also probable that some of the difference could be explained on nutritional grounds.

Fatty Acid Composition of Milk Fat

Although it has been known for some time that human milk fat shows considerable difference from cow's milk fat, from such analytical measures as iodine value, Reichert Meissl and Polenske

numbers and saponification equivalent, it is only recently that complete analyses of the fatty acids have been made. The first full analysis made was by Bosworth (1934), and this is given, along with the more recent results of Hilditch and Meara (1944*a*, *b*), Baldwin and Longenecker (1944), and Brown and Orians (1946), in Table 19. Also given are mean values for the fatty acid composition of butterfat (cow's milk) (Hilditch and Jasperson, 1941) and human depot fat (Cramer and Brown, 1943).

Bosworth made the important observation that the linoleic acid of human milk fat is not the same as that in cow's milk fat, but contains seed fat linoleic acid (*cis-cis* $\triangle^{9,12}$ octadecadienoic acid). Hilditch and Jasperson (1939, 1945) have shown that the linoleic acid of cow's milk fat is not of this form, but may be *cis-trans* or *trans-cis* $\triangle^{9,12}$ octadecadienoic acid. As can be seen from Table 19, human milk fat is almost completely lacking in the lower saturated fatty acid homologues, but this was already known from the Reichert Meissl and Polenske numbers. These two major peculiarities of human milk fat led Hilditch and Meara (1944*a*) to conclude that breast milk fat resembles a typical margarine fat blend more than it does cow's milk fat. This resemblance had already been pointed out by Polonovski *et al.* (1932), who suggested that investigation be made of the influence of diet on milk fat. No complete investigation has been made, but Polonovski (1933) found a definite increase in the Polenske number of the fat of milk taken after the subject had received a glucose supplement ; this rise, from 1·5 to 5·0, indicates an increase in the water-insoluble volatile fatty acids.

Borsarelli (1933) found an increase in the Reichert Meissl number from 2·5 to 2·7 and in the Polenske number from 0·6 to 0·7 during menstruation. This indicates an increase in all the volatile fatty acids and if this effect is real it ought to be taken into account in any study of dietary effects such as Polonovski's.

It is unfortunate that no record is available of the diets, even immediately before parturition, of the women from whom the milk was obtained. The nearest approach to such a record is Bosworth's statement that his subjects " were living in hospital at the time the milk was collected and were receiving a varied diet

TABLE 19

FATTY ACID COMPOSITION OF HUMAN MILK, COW'S MILK FAT AND HUMAN DEPOT FAT

Source / Iodine Value and Fatty Acid percentages by Weight	Bosworth[3] (1934)	Baldwin and Longenecker (1944)			Hildritch and Meara (1944a, b)						Brown and Orians[4] (1946)		Hilditch and Jasperson (1941)	Hilditch and Thompson (1936)	Cramer and Brown (1943)
						(a)			(b)				Cow's Milk Fat		
		1st and 2nd days	3rd day	Mature	Early (Fatty Acids)	Early (Fat)	Full (Fatty Acids)	Late (Fat)	Composite Sample (Fat)	Mean of 5 Preceding columns	Analysis 1	Analysis 2	Average Values	After Cod Liver Oil	Human Depot Fat
Iodine value	56·22	—	—	—	56·0	52·1	54·7	48·2	60·1	54·2	61·7	61·7	40·3	51·7	67·7
Butyric	—	0·2	0·3	0·4	—	—	—	—	—	—	tr ?	tr ?	3·6	2·0	—
Caproic	tr.	0·1	0·1	0·1	—	—	—	—	—	—	—	—	1·9	0·6	—
Caprylic	0·2	0·8	0·1	0·3	—	—	—	—	—	—	tr.	0·1	0·7	0·9	—
Capric	1·9	3·5	0·9	2·2	2·7	0·8	1·7	0·5	1·4	1·4	1·4	2·1	2·1	1·3	—
Lauric	5·8	0·9	2·6	5·5	5·1	6·1	6·4	7·0	5·9	6·1	5·4	4·8	2·4	4·1	0·3
Myristic	13·5	2·8	4·9	8·5	8·1	10·8	7·6	13·9	7·9	9·7	6·7	6·6	9·0	6·7	3·5
Palmitic	23·7	24·6	27·8	23·2	22·5	24·6	22·4	24·1	23·0	23·3	21·0	21·6	26·9	23·2	25·0
Stearic	2·6	9·9	7·7	6·9	8·3	7·3	9·0	9·6	7·0	8·2	6·8	6·8	11·0	8·2	7·0
Arachidic	0·2	4·9	2·7	1·1	1·0	1·8	0·9	—	1·1	1·0	See [1]	See [2]	1·7	0·6	—
Decenoic	tr.	0·2	0·2	0·1	tr.	tr.	tr.	tr.	—	tr.	tr.	tr.	0·1	0·5	—
Dodecenoic	—	0·1	0·1	0·1	0·1	0·1	0·1	0·1	0·2	0·1	0·1	tr.	0·2	—	—
Tetradecenoic	0·5	0·1	0·2	0·6	1·3	0·4	0·5	0·9	0·7	0·8	0·7	0·4	0·7	1·4	0·4
Hexadecenoic	4·1	1·8	2·9	3·0	3·1	3·3	3·7	2·8	5·3	3·6	2·7	2·6	3·5	3·3	0·7[5]
Oleic	37·1	36·0	37·1	36·5	36·4	32·8	36·6	30·2	35·9	34·3	39·1	39·5	31·2	42·8	45·9
Octadecadienoic	9·2	7·5	6·2	7·8	7·9	6·3	8·2	5·5	7·8	7·1	10·3	10·4	3·7	5·1	9·6
Octadecatrienoic	—	0·3	0·3	0·4	—	—	—	—	—	—	0·5	0·5	—	—	—
Octadecatetraenoic	—	—	—	—	—	—	—	—	—	—	0·2	0·2	—	—	—
Arachidonic	—	1·8	1·6	0·9	—	—	—	—	—	—	0·8	0·8	—	—	—
C_{20} and above (unsaturated)	1·1	4·6	4·7	2·4	3·5	5·7	2·9	5·4	4·1	4·3	4·3[1]	3·6[2]	1·4	5·1	1·2

[1] Both saturated and unsaturated. Includes 1·6 per cent. C_{20}, 1·7 per cent. C_{22}, 1·0 per cent. C_{24}.
[2] Both saturated and unsaturated. Includes 1·1 per cent. C_{20}, 1·9 per cent. C_{22}, 0·6 per cent. C_{24}.
[3] Fatty acids computed from Bosworth's (1934) data and given by Hilditch and Meara (1944*a*).
[4] Two batches of esters prepared from 1 sample of fat.
[5] As arachidonic.

of fruit, vegetables, meat, fish, milk, salads and iodine and cod liver oil when necessary ".

In cows the feeding of certain fats does produce changes in the fatty acid composition of the butter (see numerous papers by Hilditch and his collaborators) ; this effect consists largely in the alteration of the normal distribution of fatty acids, particularly the depression of the amount of the lower saturated homologues, with a proportional increase in oleic acid. In some cases abnormal fatty acids may appear in the milk, *e.g.*, erucic acid appears in the butter when rape oil is given.

Brown and Orians (1946) claimed from their results a relationship between human milk fat and depot fat. Here again the seed fat form of linoleic acid predominates in the linoleic acid mixture. This observation does not advance the solution of the problem, for it is well known that abnormal dietary fats can be deposited in the depot fat, and so this acid may reach milk fat directly from the dietary fat or indirectly through the depot fat. Although the accumulation of abnormal fatty acids in the body fat of animals is well known, there are several anomalies which might also apply to their appearance in milk fat. Hilditch *et al.* (1942), working on the depot fats of wild animals, found that lion and cat fats contained lauric acid, which might have been obtained from the flesh of animals eating coconut cake which they, in turn, consumed ; on the other hand, the Somali sheep, known to have consumed coconut cake, showed no lauric acid. Such anomalies indicate that although the fatty acid composition of milk fat may be influenced, directly or indirectly, by diet, the effect is unlikely to be simple.

Non-Fat Lipids

All the work so far described was on the gross lipid content of the milk. This, as well as covering triglyceride true fat, also includes phospholipins, cholesterol, cholesterol esters and a certain amount of unsaponifiable material.

Phospholipins have been estimated by several workers by estimation of P in the gross fat, the phospholipin being expressed as lecithin (Stoklasa, 1897 ; Burow, 1900 ; Koch and Woods,

1905 ; Nerking and Haensel, 1908 ; Glikin, 1909). There is little consistency between the results of different workers, which vary from Nerking and Haensel's values, which give a mean and S.E. on 10 estimates of 499 ± 5·7 mg. per cent., to Stoklasa's values of 170 to 186 mg. per 100 ml. It is impossible from the data available to apportion the sources of this variation ; differences in analytical method probably account for a great deal of it. There is no record given of stage of lactation. Nerking and Haensel give also the lecithin estimated in the milk of cow, ass, ewe, mare and goat, and there is some indication that between species there is an association between gross lipid content and phospholipin content of milks, but the values found do not suggest that this holds within species.

The cholesterol content of the milk has also been studied by several workers but the method of estimation in some of the earlier studies has been shown to be unsatisfactory. The main work on this component has been done by Denis and Minot (1918), Wacker and Beck (1921), Fox and Gardner (1924), and Mühlbock (1934). Even among these, differences in methods of estimation appear to have produced considerable effects on the results. Thus the mean values derived from the results of these authors are 19·7 ± 0·9, 13·9 ± 1·1, 29·0 ± 1·7, and 11·06 ± 0·37 mg. per 100 ml., respectively.

Fox and Gardner (1924) claimed that total cholesterol declines in the first few days of lactation. Some of the evidence adduced might be explained by differences introduced by combining the results of several authors using different methods of estimation, for Fox and Gardner's results are overall much higher than the others at all stages of lactation and it is from their results that 19 out of 21 of the estimations for the first days of lactation come. However, the claim is supported by data given on the milk of 3 women, obtained at three different stages of lactation, in which the mean total cholesterol was 50 per cent. higher (41 mg. per 100 ml.) on the 6th day than in the two later sets of samples (26 mg.). The claim receives more convincing support from Mühlbock's (1934) data, where the total cholesterol for the 4th to the 10th days is found to be 26·0 ± 2·5 and that for the 12th to the 47th days 11·06 ± 0·37 mg. per 100 ml. Fox and Gardner

associated the high initial cholesterol with the high blood cholesterol during pregnancy.

Cholesterol is present in both free and ester form, the ratio of free to ester, as found by Fox and Gardner, being, in general, greater than 1 (0·96 to 3·57). Mühlbock found a much higher ratio, 2·7 to 161 ; these high ratios suggest that cholesterol esters are present in negligible quantities and so it seems that they may on occasion be completely absent. Beumer (1918) stated that all the cholesterol of milk was in ester form, but in 1922 he found a sample of human milk with a ratio of 1·24 for free to ester form.

In none of these investigations was the sampling procedure satisfactory, and few sources of systematic variation were examined. Variation between different stages of a nursing was found by Fox and Gardner to be considerable, but it showed no tendency to alter systematically. They also found great differences in cholesterol content between the milks taken at the same time from the two breasts. This latter finding, combined with the great variation in total cholesterol content (recorded range 13 to 47 mg. per 100 ml.), suggests that some of the cholesterol may be merely incidental in the milk and have no normal physiological significance for the infant.

The total unsaponifiable matter in the milk has a mean value of 150 $\pm$ 14 mg. per cent. (Fox and Gardner), of which about a fifth is accounted for by total cholesterol. The remainder is described as " sticky yellow oils " and resinous matter produced during saponification.

Lactose

Of all the components of human milk, lactose seems to be the most constant. The variation about the mean is large, as can be seen from the histogram, Fig. 10, based on 1010 samples (mean 7·23, S.D. 0·67), but the coefficient of variation is very much less than that of protein, 9 per cent. as against 24 per cent., and there is no indication that such variation as exists arises from any systematic cause. The mean and S.D. of lactose estimations in 586 samples of mature milk calculated from Kon and Mawson's (1950) data are 6·93 and 0·34 g. per 100 ml., giving a C.V. of 5 per cent. No indication has been found of diurnal variation, Fig. 1,

but Novellis di Coarazze (1936) reported a diminution and later partial recovery during nursing. There are slight indications of individual trends (Denis and Talbot, 1919 ; Deem, 1931), but in no investigation has this been found to be statistically significant, and from the small magnitude it is unlikely to be physiologically significant.

Variation with stage of lactation has not been claimed for mature milk apart from a terminal decline, but there does appear to be a rapid rise in lactose content in the first few days of lactation. Widdows *et al.* (1935) found a value of about 3 g. per 100

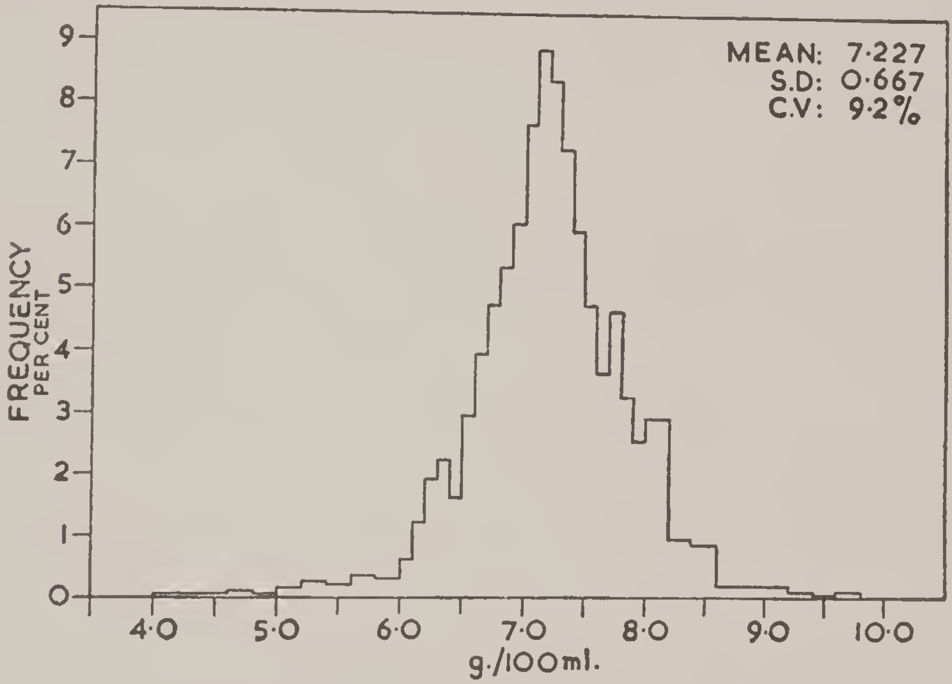


FIG. 10 DISTRIBUTION OF LACTOSE CONTENT

ml. for antenatal secretion and the secretion of the 1st day, about 7 g. on the 5th day, and 7·3 on the 8th day (Fig. 7). Woodward's (1897) results indicate earlier attainment of the mature value, at about 3 days, and those of Castellanos and Lizarralde (1943) and Escudero and Sola (1943) later attainment. If 10 days be accepted as the period for attainment of mature milk, there is, thereafter, no perceptible trend up or down.

Day-to-day Variation

This appears to be the greatest source of variation in lactose content and is prominent in Schlossmann's (1902) data, where the day to day variation makes up 80 per cent. of the total.

Effect of Diet

The greater part of the evidence available indicates that the lactose content of the milk remains constant in spite of changes in the diet, and this is the view generally held (see, *e.g.*, Kon and Mawson, 1950), but there are a few anomalous records which indicate that the lactose content may, under certain circumstances, be influenced by the diet. Deem (1931) found a significant difference in lactose content of the milk of her subjects on different diets (Table 2), the highest lactose content appearing on the institution diet and the lowest on the high-fat diet. Escudero and Pierangeli (1940-41*a*), however, relating lactose of the milk to carbohydrate intake in g. per kg. theoretical maternal weight, found no effect, but the same authors (1940-41*b*) recorded a rise in the lactose content of the milk obtained at their " ginegaladosia " in 1939-40 when the diet of the donors was supervised, over that in 1938 when the diet was not supervised. The rise was from 6·7 to 7·1 g. per 100 ml., commensurate with that found by Deem of 7·3 to 7·7 g. per 100 ml. Polonovski's (1933) records of increased fat content of the milk on a glucose-supplemented diet showed also that the lactose content of the milk did not change, but the lactose content of the milk does not necessarily alter with the carbohydrate intake and may simply alter with a general improvement in diet.

The detailed results of Escudero and Pierangeli (1940-41*a*) for lactose classified according to other components of the diet, protein and fat, are given in Table 13. There is no apparent relation between the levels of these in the diet and the level of lactose in the milk.

It has been claimed, in animals (Foa, quoted by Meigs (1922)), that the amount of lactose in the milk secreted bears a quantitative relation to the blood glucose. If this were true, the milk from the nursing immediately after the post-absorptive rise in blood sugar might be expected to show a high lactose content, but there is no evidence for this in the form of any diurnal variation in the lactose content of milk. Blood sugar variations are, of course, not a simple function of carbohydrate absorption. Eliminating, to some extent, the effect on blood sugar of absorption from the gut, Tolstoi (1935) examined the effect on milk lactose content of

varying the blood sugar of lactating diabetic women by means of insulin. He found no correspondence between the lactose content of the milk and the blood sugar level.

Under normal circumstances the blood sugar, apart from post-absorptive changes, is almost constant and independent of diet, and so if milk lactose is a direct product of blood sugar, as it would appear to be (Meigs, 1922), there is little reason to expect the lactose content to vary with diet. Such indications as there are that milk sugar may vary with diet suggest, therefore, that dietary components may alter the efficiency of conversion of blood sugar to milk lactose. This is suggested by the work of Sato *et al.* (1948), who found that deficiency of vitamin B_1 altered all milk components except urea and vitamin C.

A bar to combining all the observations made on lactose content is the variation in method of estimation. Most of the available figures have been obtained by one of the reduction methods, but many estimations have been made colorimetrically and many values for lactose are simply derived by difference. Macy (1949), in a collected table of most of the work on milk composition done by her and her co-workers, gives mean values for lactose in mature milk as estimated directly and as estimated by difference, of 7·1 and 6·8 g. per 100 ml., the difference having a standard error of 0·03 ; these means are based on 198 and 313 estimations, respectively. Folin and Denis (1918) compared the values for lactose found by colorimetric (picrate) and titrimetric (copper sulphate reduction) methods and found no significant difference. Different reduction methods give different results, and these differences have been used by Polonovski and co-workers (1931, 1933) to examine the different forms of sugar in milk. Reduction of copper sulphate gives the lowest value for reducing substances and appears to represent true lactose ; permanganate reduction gives a higher value and is believed to estimate two other sugars, called by Polonovski allolactose and gynolactose, which may be precursors of true lactose ; and iodimetric titration gives a still higher value and is claimed to include also a further sugar moiety normally forming a glycoside with the milk protein. This total extra reducing power when estimated iodimetrically appears to amount to about 10 per cent.

of the lactose as normally estimated by copper reduction methods, but the reducing power of the allolactose and gynolactose isolated and described by Polonovski and Lespagnol (1931*b*, 1933) is very much less than that of lactose, about one-fifth, so that the recorded additional reducing power found iodimetrically may represent as much as 50 per cent. additional substance considered as sugar. This, however, does not agree with the discrepancy shown by Macy (1949) between the values found for sugar by direct estimation and by difference, unless we assume that all these extra sugars are normally in combination as protein glycosides and are thrown down with the protein in protein precipitation before estimation of sugar. Husset-Bierby (1943) regards all these different sugars as being in combination with the milk protein. It would be interesting to know if these sugars vary with protein content ; they appear to show no diurnal variation, nor do they vary with the true lactose content (Polonovski *et al.*, 1931).

It is difficult to know what the significance of these extra bound or free reducing sugars is, but failing further information the suggestion of Husset-Bierby that they are the precursors of true lactose and represent intermediaries between blood glucose and milk lactose appears plausible.

Habild (1949) reported that milk as secreted contains an equilibrium mixture of α- and β-lactose, so it is not to be expected that any nutritional advantage would be gained by using the β- form for infant feeding.

Minerals

Total Ash

Macy (1949) gives a mean of 202·0 $\pm$ 0·91 mg. per 100 ml. for 390 samples. This is, in general, higher than that recorded by other authors. Escudero and Repetto (1944) record a mean of 188 mg. and Telfer (1924) of 196 mg., but Macy's series is much larger than either of these. Escudero and Rothman (1949) found a mean of 202 mg. per 100 ml.

Although Macy gives only an overall mean for ash of mature milk, an earlier publication (Nims *et al.*, 1932*b*) gives strong evidence showing a steady decline of ash throughout lactation. Escudero and Repetto also show evidence of a decline

in ash throughout lactation, but the decline they show is less definite.

There is general agreement that the total ash declines very rapidly in the first few days of lactation (*cf.* ash components in Fig. 11). Macy gives means for the ash of the first and second 5 days' colostrum as 308 and 267 mg. per 100 ml. Widdows *et al.* (1935) found the ash of the antenatal secretion to be high and fairly constant, with a sharp rise at labour and thereafter a steady decline. Castellanos and Lizarralde (1943) show no definite trend in ash content of milk during the first 5 days, but only a general high level of ash, with a mean of 350 mg.; nor do these authors show the trends found by others in the separate components of the ash.

Calcium

Macy (1949) gives a mean of 34·4 ± 0·27 mg. per 100 ml., derived from 628 samples. This is considerably above the values recorded by most other workers: 29·9 ± 0·5 (Kon and Mawson, 1950); 28·46 ± 0·17 (Winikoff, 1944; Ritchie, 1942) for Australian women; and 22·4 ± 0·5 mg. for Japanese women (Uga, 1935). Drummond *et al.* (1939) for 20 random samples, not always for 24 hours, record a mean of 31·2 mg. per 100 ml. Part of the discrepancy between the results of different workers may be due to inadequate sampling.

There appears to be no significant diurnal variation (Hunaeus, 1909; Winikoff, 1944), but Stransky (1926) shows a fall from fore to after milk of 57 to 21 mg. Ca per 100 ml. Macy *et al.* (1931) and Lowenfeld *et al.* (1927) found no significant change in Ca between fore and after milk. If there is a real change during nursing it may mean that some of the discrepancy between the results quoted above is caused by inadequate sampling, but it seems unlikely that this would cause such large differences as exist between, for example, Macy's and Uga's figures.

Variation with Stage of Lactation

The evidence for a change from colostral to mature milk is inconsistent. Macy (1949) gives, for the first and second 5-day colostral periods, means of 48·1 ± 2·3 and 46·4 ± 1·4 mg. per

100 ml., respectively, based on 28 and 46 samples. Castellanos and Lizarralde (1943) and Lowenfeld *et al.* found no change during the colostral period. Winikoff found a general rise in Ca from the 1st to the 10th day *post partum* in 4 out of 5 subjects, the

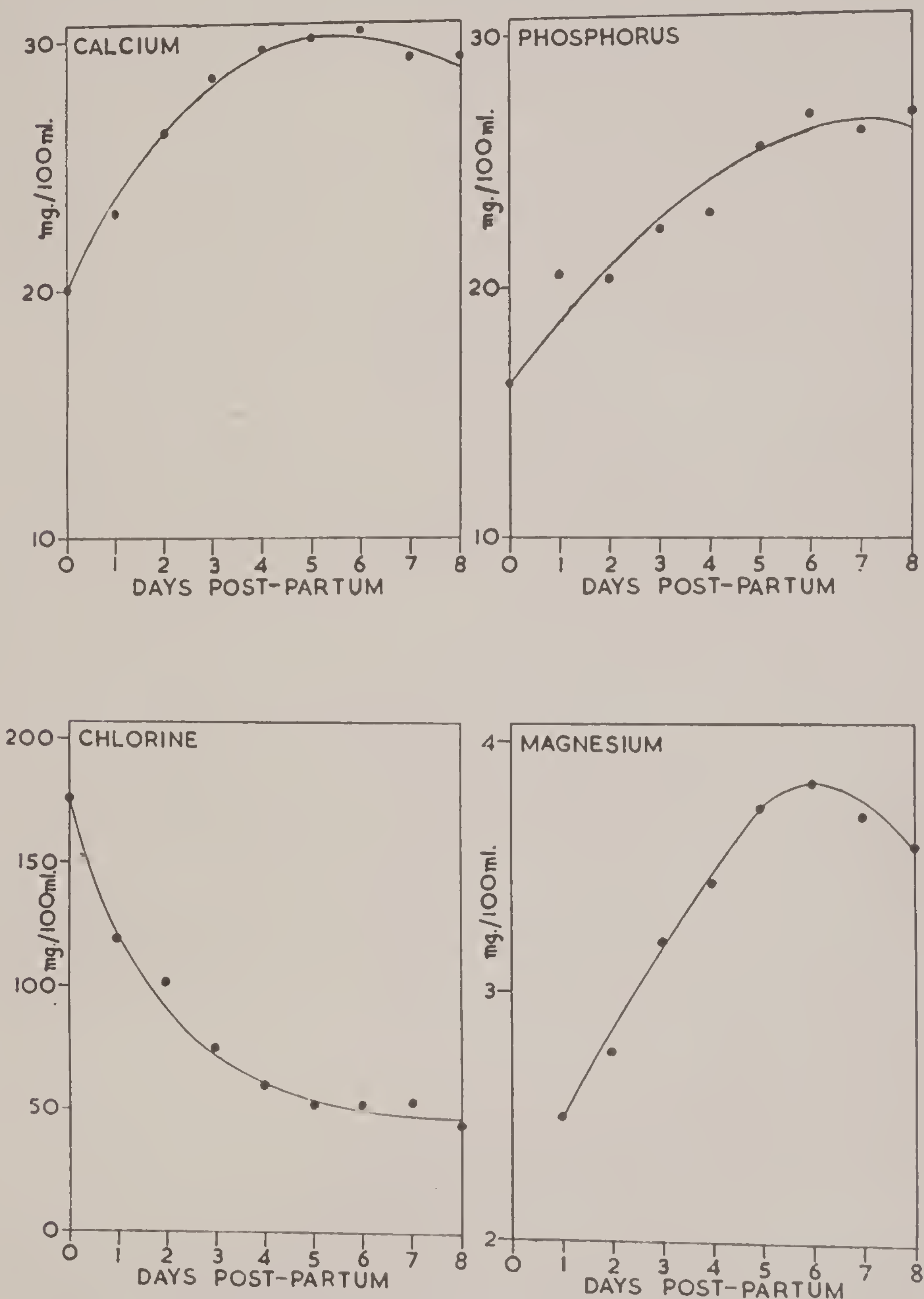


FIG. 11 MINERAL CONTENT OF COLOSTRUM
(Ca, P, Cl, Widdows *et al.* (1935); Mg, Winikoff (1944).)

5th subject showing an almost constant Ca level. On the other hand, the same study shows a generally lower level of Ca on the 9th than on the 7th day of lactation in 6 other subjects and also shows a general mean for colostrum of 28·2 ± 0·4 mg. per 100 ml. Widdows *et al.* (1935) found a rapid rise from the antenatal level, about 22 mg., to about 30 mg. from the 3rd day *post partum*, after which the level remained constant. It appears then that the individual variation is so great as to obscure any average trend.

The evidence for later alteration in Ca content is more definite. Winikoff's results show a rise in Ca content from the 1st to the 3rd month with a constant decline thereafter (Fig. 12). Holt *et al.* (1915) found a fall in colostrum followed by a rise to early mature milk and a second fall to late mature milk. Ritchie's results are shown with Winikoff's in Fig. 12 ; they show the same trend when considered separately. Nims *et al.* (1932*b*) also show a maximum between the 6th week and the 6th month. This maximum is of interest in relation to the calculated daily requirement of Ca in the infant, which rises to a maximum in the 3rd month (Clements, 1942).

Variation with Parity

In Fig. 12 are given the curves of variation of Ca content with parity found by Ritchie. No measure of dispersion was given by Ritchie, so it is impossible to examine the significance of this variation, but the form of the curves is interesting in that it follows the general pattern of reproductive efficiency, which tends to increase to a maximum at the second or third pregnancy and to decline in later pregnancies. Sikes (1906*a*) found no significant effect of parity, but he considered only primiparae as compared with multiparae, so that a change of the type found by Ritchie would have been masked.

Day-to-day Variation

The only work giving Ca values in such a form that this variation can be adequately examined, that of Hunaeus (1909), shows that the day-to-day variation is small, with a maximum difference of about 1·5 per cent.

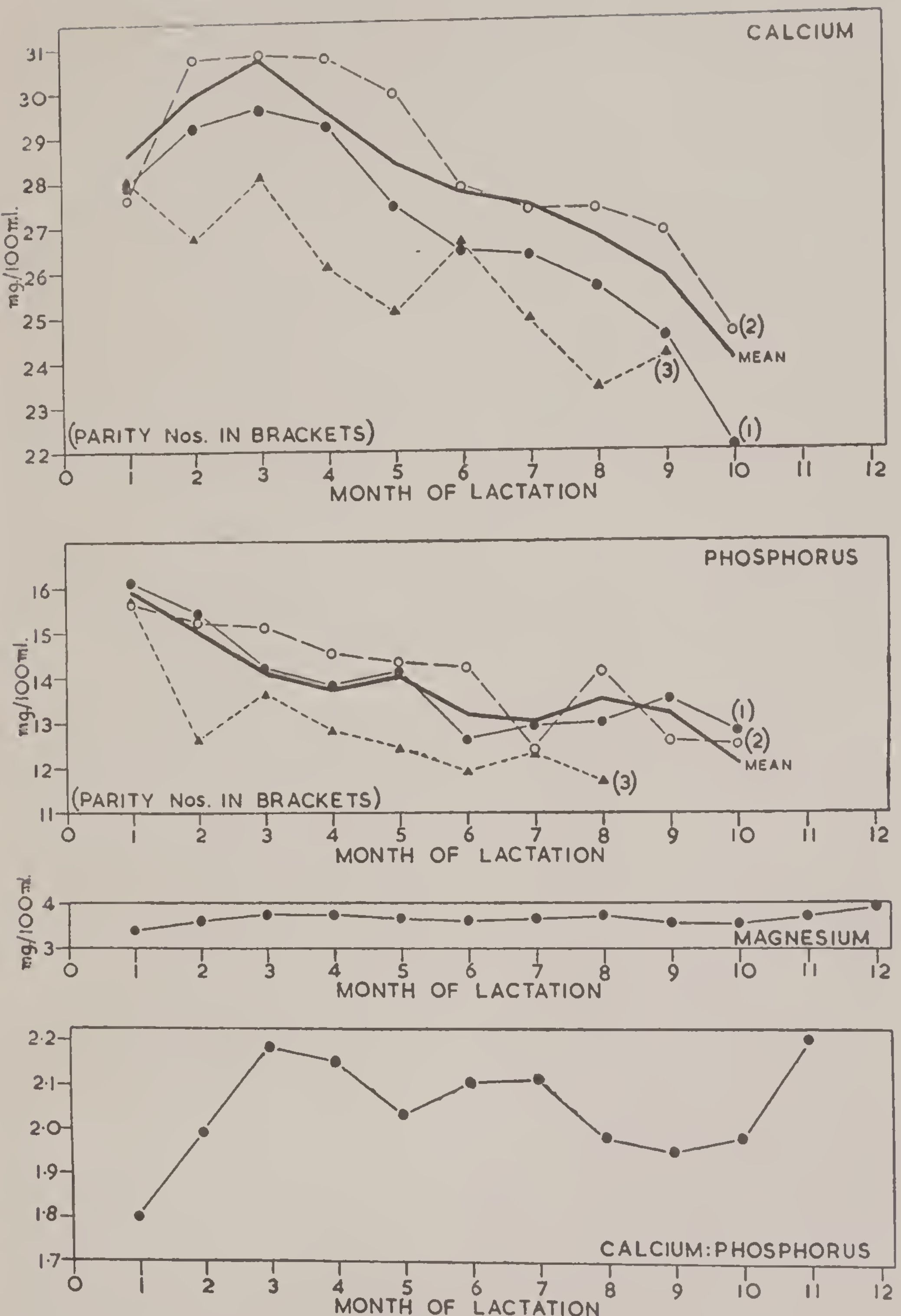


FIG. 12 VARIATION OF MINERAL CONTENT WITH STAGE OF LACTATION (Ca and P, Ritchie (1942). Ca and P means from Winikoff (1944). Ca : P ratio calculated from Winikoff's means. Mg, Winikoff (1944).)

Individual Variation

Like the energy-yielding components of milk, Ca appears to follow individual trends. This is apparent from the colostrum data, and from those of Nims *et al.* (1932*b*) and Hunaeus (1909).

Effect of Diet

This source of variation has not been subjected to any systematic study. Studies on the Ca content of the milk of mothers of rachitic and non-rachitic infants are inconclusive. De Buys and von Meysenbug (1924) found the former to have a lower Ca, 27·5 mg., than the latter, 32·6 mg. per 100 ml., but Telfer (1924) found no significant difference between two such groups. Nor did Telfer (1930) find any difference in the Ca content of milk of groups described as "industrial town dwellers", "healthy women" (country dwellers) and "poorly nourished women" (town dwellers). Ritchie found means of 27·1 mg. and 27·9 mg. for milk of mothers of rachitic and non-rachitic infants. It is not possible from Ritchie's results to examine the significance of this difference, but it is very small.

Burhaus and Smith (1923) claimed a racial difference between white and coloured mothers, the mean Ca for the former being $29{\cdot}7 \pm 1{\cdot}04$ and for the latter $25{\cdot}5 \pm 0{\cdot}96$ mg. per 100 ml. It might be attractive to believe that this difference is due to nutrition of the groups rather than to race, but the researches on rachitic and non-rachitic children added to the evidence given by Uga (1935) on Japanese women make it seem possible that there may be a true racial difference.

The effect of administering Ca was investigated by Hunaeus (1909) with negative results, but Ritchie (1942) showed that although Ca lactate given alone to the mother had no effect on the Ca content of the milk, given with vitamin D it raised the Ca content by as much as 25 per cent., and Herz (1933) claimed that a change from a Ca-poor to a Ca-rich diet increased milk Ca by 8 to 15 per cent. Toverud and Toverud (1931) make a similar claim. Ritchie treated only a small number of women whose milk was by normal standards low in Ca, and there is no indication whether there is a limiting value of Ca in milk above which no increase can be produced by such medication. Such informa-

tion would be of interest in view of Clements's (1942) calculation that with the average Ca content of milk, an average volume and an absorption efficiency of 60 per cent., an infant will receive inadequate Ca for the first 12 weeks of life.

Uga's (1935) investigation of the Ca content of milk, although probably distorted by inadequate sampling, showed, in general, very low values for Ca in the milk of Japanese women. He also claimed that the Ca values were closely correlated with the peroxidase (Arakawa) reactions.

	Positive peroxidase	*Intermediate*	*Negative*
Ca, mg. per 100 ml. .	26·52 ± 0·92	21·09 ± 0·48	13·22 ± 1·10

The significance of this finding is doubtful. Obviously the differences between the Ca contents of the different peroxidase-response groups are significant, but the response to the test is only roughly quantitative and the test itself is of notoriously low specificity. A peroxidase has been shown to be present in cow's milk and has been isolated therefrom, but it can be inhibited by many substances. We do not know just what association Uga found ; he appeared to believe that, since this peroxidase reaction has been shown also to be associated with vitamin B intake, the Ca level of the milk might be modified by the intake of this vitamin.

Turner and Weeks (1934) found the Ca content of the skimmed milk of women from a goitre area to be higher than that of women from a non-goitre area in the early months of lactation, but lower after the 5th month.

It appears likely that, although in some cases diet may have an effect on the Ca content of the milk, the intake of the child will be influenced more by volume of milk consumed and by dietary effects on the absorption and utilisation of Ca by the child. It is noteworthy that the absolute absorption of Ca by the infant fed on cow's milk is greater than that on breast milk, but the percentage absorption of the Ca (and of the P) of breast milk is greater, 62 as compared with 50 per cent. for Ca and 83 as compared with 81 per cent. for P (Wang *et al.*, 1924 ; Witt, 1932).

Phosphorus

Data on the P content of the milk are fewer than those on Ca and are complicated by the several forms of P. Macy (1949)

gives a mean corresponding to her Ca mean for 628 samples, of 14·1 ± 0·1 mg. per 100 ml. for total P in mature milk. This is in general agreement with the values of most other authors : 13·0 ± 0·22 mg. (Kon and Mawson, 1950), 13·93 ± 0·08 (Winikoff, 1944 ; Ritchie, 1942), 17·54 ± 0·84 (Burhaus and Smith, 1923) ; 14·2 (Drummond *et al.*, 1939).

Burhaus and Smith, on some 50 subjects, partition the P, giving 5·15 ± 0·3 for inorganic and 12·39 ± 0·9 mg. per 100 ml. for organic P, making inorganic about 30 per cent., of the total. Sikes (1906*a*) found non-protein P to be of the order of 60 per cent. of the total, while Plimmer and Lowndes (1937) found it to be about 80 per cent. von Meysenbug (1922) gives a mean figure for " inorganic phosphate " of 4·08 mg. for mothers with normal infants and 4·61 mg. for mothers with rachitic infants.

Calculation of P partition from the average casein and phospholipin content of the milk gives the following relation :

Organic P — Total P — Inorganic P

Casein P — Phospholipid P

5 mg. — 7 mg. — 14-16 mg. — 2-4 mg.

Lenstrup (1926) gives the following scheme for the partition of milk P :

Total P

Acid-soluble P — Acid-insoluble P

Inorganic P — Organic P — Lipoid P — Protein P

His average values for these fractions are given in Table 20.

TABLE 20

Phosphorus Partition in Human and Cow's Milk

(mg. per 100 ml.) (Lenstrup, 1926)

	Total P	Acid-insoluble P	Acid-soluble P		
			Total	Inorganic	Organic
Human milk . .	14·2	2·6	11·6	5·1	6·5
Cow's milk . .	95·4	17·1	78·3	67·1	11·1

Bomskov (1932) presents a similar, but extended, partition from the analyses of about 30 milk samples from 3 women.

TABLE 21

PHOSPHORUS PARTITION IN HUMAN MILK

(Bomskov, 1932)

Fraction	Total P	Acid-insoluble P	Acid-soluble P				
			Total	Inorganic	Pyro P	Hexose ester P	Remaining P
P mg./100 ml.	14·54±0·22	2·82±0·28	12·07±0·24	5·57±0·20	0·65±0·10	0·75±0·13	5·08±0·18
No. of samples	32	30	33	35	30	29	30

Hochheimer (1933) found for total, acid-insoluble, acid-soluble, organic and inorganic P, 12, 2, 10, 5 and 5 mg. per 100 ml., respectively. De Toni and Graf (1938*b*) quote their own data (1938*a*), for inorganic P, 5·3 mg. ; for hexosephosphoric ester P, 0·6 mg. per 100 ml.

These findings agree fairly closely with each other but bear little relation to the theoretical values for organic P calculated above from lecithin and casein P. It would appear from this that either acid-insoluble P is not in fact an adequate representation of lipoid and protein P, or else the average values for casein and phospholipin, or for the P content of casein, are inaccurate. It may be that some of the lipoid and casein P is included in Bomskov's unspecified remainder. Mellander (1945, 1947) studied the distribution of P in the fractions obtained by electrophoretic analysis of casein and gives a value of 0·47 per cent. P in casein. Mandel and Bieth (1948) give casein P in human milk as 1·3 mg. per 100 ml., figures with approximate correspondence.

Both Leontev (1948) and Mandel and Bieth found in human milk ribonucleic acid P 0·25 mg. per 100 ml. and no deoxyribonucleic acid. This compares with 1·6 and none for cow's milk.

There does not appear to be any significant diurnal variation or variation from beginning to end of a nursing in the P content, although Macy *et al.* (1931) claimed that P increases from beginning to end of a nursing, with a corresponding decrease in the Ca : P ratio.

Variation with Stage of Lactation

Winikoff (1944) shows a general tendency for P to increase in the first few days of lactation and to reach a constant level by about the 7th day *post partum*, and the same tendency is shown by Widdows *et al.* (1935) (Fig. 11), but again Castellanos and Lizarralde (1943) show no definite tendency during the colostral period. There is general evidence for a steady decline in P content of mature milk throughout lactation, this being best shown by the graph of Winikoff's and Ritchie's data combined (Fig. 12). Presumably the P content passes through a maximum somewhere in the 1st month, but as none of the colostrum data extend beyond 10 days the position of the maximum cannot be determined precisely. It seems possible that it may occur at 10 to 14 days *post partum*.

Because of the decrease of both Ca and P throughout lactation the Ca : P ratio does not alter much (Fig. 12). Such major irregularities as do occur are probably due to the fact that the Ca maximum lags behind the P maximum by about 2 months.

Variation with Parity

In Fig. 12 are shown Ritchie's data for P corresponding to the curves derived from his data for the variation of Ca with parity. The same tendency is apparent, but the trends are not quite so well defined.

Individual Variation

Again a strong tendency to individual trends can be seen in the values for both colostrum (Winikoff) and mature milk (Nims *et al.*, 1932*b*).

Effect of Diet

As with Ca, the studies on the P content of the milk of mothers of rachitic infants are inconclusive. Telfer (1924) and Ritchie (1942) estimated P in the milk of mothers of rachitic infants, but found no convincing evidence of a difference.

The data of Burhaus and Smith (1923) on the milk of white and coloured women are of interest in that they show the " inorganic P " to be significantly higher in the white (5·5 mg. per

100 ml.) than in the coloured (4·8), although the total P is slightly lower.

Turner and Weeks (1934) found, as with Ca, that the inorganic P content of the skimmed milk of women in a goitre area was higher at the start than that of women of the non-goitre area, but fell below it after about 4 months. This finding for both Ca and P may have been dietary in origin or possibly an indirect effect secondary to mild endocrine disturbance.

Magnesium

Macy (1949) gives a mean of 3·5 ± 0·04 mg. per 100 ml. This is close to Winikoff's (1944) mean of 3·39 ± 0·04, although Winikoff regarded this as being a lower limit when compared with the estimates of other workers. Certainly the older workers quoted by Czerny and Keller (1923) reported values of the order of 5 mg., and Holt *et al.* (1915) and Telfer (1930) found means of about 3·6 and 4·2 mg., respectively ; but it is not impossible that these high results were due to poor technique. Hasegawa (1937), in a paper primarily on technique, found a value for Mg of 3·8 ± 0·27 mg. from 12 samples.

Winikoff found no diurnal variation in Mg content of milk, although the difference in Mg content of the milk at successive nursings could be as great as 15 per cent.

Variation with Stage of Lactation

Winikoff's colostrum data show a rapid rise in Mg content from parturition to about the 4th or 5th day of lactation, and then an irregular plateau. The mean for the total of her colostrum values is 3·22 ± 0·07 mg., which indicates that there may be a rise from colostrum to mature milk. There is no evidence of a decline in the Mg of mature milk with progress of lactation ; the values are remarkably constant (Fig. 12). Holt *et al.* (1915) show, after a high colostrum value declining to transition milk (12 to 30 days), a high content in early mature milk and a second decline. Macy (1949) shows a high value, 4·2 ± 0·24 mg., for the first 5-day colostrum period, but in the second 5-day period a value identical with that for mature milk.

There is no apparent reason for the consistent difference between Macy and Winikoff in the observed content of minerals, the former showing a colostral fall and the latter a colostral rise in Ca, P and Mg.

Chlorine

Macy (1949) gives a mean of 37·5 ± 0·6 mg. per 100 ml. for Cl. This is very much less than Sisson and Denis's (1921) mean of 54·0 ± 0·79, but is comparable with most other estimates. Apart from higher values in the first fortnight (which are not included in the above mean), Sisson and Denis were not able to find any definite trend with stage of lactation. Holt *et al.* (1915) show a rise in the Cl of very late milk, from 36 to 44 mg. per 100 ml.

The colostral Cl shows a rapid decline for about the first fortnight ; Macy gives 58·6 and 45·7 mg. per 100 ml. for the first and second 5-day periods, but these are below the values for colostral milk found by other authors. Holt *et al.* give a mean of 57 mg. for the first 12 days, and Sisson and Denis 74 mg. for the first fortnight. Widdows *et al.* (1935) show a rapid decline (Fig. 11) in the first 8 days of lactation, after an irregular high level in the prenatal secretion.

Macy *et al.* (1931) found no change in Cl content between fore and after milk, but there is a suggestion in their data of a diurnal variation, with a minimum in the evening about 6 to 10 p.m. ; this is based, however, on samples from only 2 subjects.

Ishii (1938) found great variation in the Cl content of the milk from the two breasts, sometimes as great as 100 per cent. He confirms the general finding that Cl has a high variation and gives a mean of 39·6 ± 0·91 mg. per 100 ml. (C.V. = 39 per cent.). Ishii also claims a relation between the peroxidase reaction and the Cl content, the Cl being less with a positive reaction. A similar but less pronounced relation was found to hold between the peroxidase reaction and the blood Cl, the net result being that the ratio blood Cl : milk Cl declined with decreasing strength of the peroxidase reaction.

Burhaus and Smith (1923) found Cl to be high in the milk of

coloured subjects, 57 mg. as compared with 46 mg. in white subjects.*

SODIUM

Macy's (1949) is the only major series available and gives a mean of 17·2 ± 0·26 mg. from 302 samples. This mean is higher than the values found in any of the lesser series : Escudero and Rothman (1949), 12 ± 1·3 mg. on 14 subjects ; Holt *et al.* (1915), 12·6 ± 1·0 on 26 samples ; Vuk and Sándor (1937), 9·7 shortly after parturition, decreasing to 7·3 to 5·4. Goldmann (1938) found a mean of 12 mg. (range 8 to 17) on 55 samples from 6 subjects with great variation between subjects ; the range of means for subjects was 10·6 to 16·5. Certainly Na, like Cl, is one of the most variable of the major mineral components, with a C.V. of 25 per cent.

Macy also indicates a rapid decline in Na during the colostral period, the means for the first and second 5-day periods being 50 and 29 mg., respectively, but the scatter in early colostrum is great, S.D. 28·0. Castellanos and Lizarralde (1943) show a doubtful decline in Na for the first 4 days *post partum*.

Pommerenke and Hahn (1943) found radio-active Na present in the milk within 20 minutes of administration. It reached its maximum concentration within 2 hours. This suggests a simple diffusion process with continuous secretion of the diffused material. The secreting alveolar epithelium of the mammary gland is commonly assumed to be apocrine in type, but the histological basis for this view may not be valid (Richardson, 1947).

* Since this book went to press Kermack and his collaborators, Miller and Jackson (1951), have published results of a study of Cl content and electrical conductivity in about 670 women. Cl and yield were inversely correlated. The mean for milk from mothers with an "adequate" (not otherwise defined) milk yield was 34·5 mg. Cl, the range 14·5 to 64·8 mg. and for milk from mothers with "inadequate" lactation the mean was 81·8 mg. and the range 26·1 to 215·1 mg. per 100 ml. Cl and duration of lactation up to 5 months also were inversely correlated. There was a considerable difference between breasts in some but not in the majority of the women. During the first month there was a diurnal rhythm with maximum about mid-day. Fore milk contained more Cl than after milk.

Potassium

Macy (1949) gives a mean of 51·2 mg., S.D. 8·5, for 18 samples, Burhaus and Smith (1923) 51·1 mg. and Escudero and Rothman (1949) 52·1 ± 3·3 mg. Holt *et al.* (1915) give a mean of 47·7 ± 1·4 mg. (26 samples), which is comparable with these, but Goldmann (1938), in milk from the same 6 subjects as yielded his Na figures, found an overall mean of 70 mg. (range 50 to 85). The range of Goldmann's individual means is 76·8 to 101·7 mg. per 100 ml. Leulier *et al.* (1937) found a mean of 55 mg. and gave evidence of diurnal variation, the level being highest at noon nursing. These authors claimed that stage of lactation does not affect the K content of the milk. There are insufficient data to test this for mature milk, but colostrum does appear to have a high K content. Macy found 74·5 mg. and 63·6 mg., S.D. 6·8, for the first and second 5-day colostral periods ; Castellanos and Lizarralde (1943) about 70 mg. in the colostrum of the first few days (but not declining) ; Holt *et al.* (1915) 77 mg. for the first 12 days and 59 mg. for the period 12 to 30 days.

Burhaus and Smith found a higher K content in the milk of coloured (53·2 mg.) than in that of white women (49·0 mg.), but the difference was small.

Iron and Copper

The range of Fe recorded in milk by different authors is from 0·02 to 0·35 mg. per 100 ml. The highest value is given by Escudero and Rothman (1949). Their general mean value is 0·1 mg., but Wallgren's (1932) mean was 0·044 mg. The same mean value holds approximately for colostrum, 0·13 mg. given by Castellanos and Lizarralde (1943).

The reliability of the estimation of amounts of Fe of such magnitude is always open to doubt, since contamination, either from the vessels in which the milk is collected, or by dust from the air, is difficult to avoid. It is probable that the high levels of Fe found by Reis and Chakmakjian (1932), mean 0·36 ± 0·02 mg. per 100 ml. on 14 samples from 7 women, are the result of contamination.

Estimations of Cu in milk are even fewer. Davidson and Leitch (1933-34) quote values from 0·04 to 0·08 mg. per 100 ml.,

and Remy (1932) gives the values 0·09 and 0·19 mg. Escudero and Rothman (1949) found a mean of 0·039 ± 0·005 mg. with maximum and minimum of 0·085 and 0·015 mg., respectively. Zondek and Bandmann (1931), in 85 samples, found from 0·05 to 0·06 mg., Hess *et al.* (1923) 0·04 and 0·06 mg. in milk from 2 subjects, and Haurowitz (1930) 0·07 mg. per 100 ml. Munch-Petersen (1950) gives the average Cu content of the milk of 10 women as 0·054 ± 0·013 mg. and found no increase when Cu was given intravenously.

Manganese

Escudero and Rothman (1949) found a trace of Mn in 5 out of 14 of their samples. De (1935) and Dingle and Sheldon (1938) found Mn spectrographically, but Drea (1938) found it in only 3 of his 6 samples.

Iodine

In mature milk the variation in I content is great and appears to depend largely on the I content of the diet. Turner and Weeks (1934) give values from 6·0 to 23·0 μg., average 12·4, on 295 samples of skimmed milk from 19 women. The milk samples were taken from two areas, a goitre area and a normal one, and the average I level of the milk was higher for the former than for the latter area. Hercus and Roberts (1927) found the I content of the milk of goitrous women to be below normal, but none of Turner and Weeks's subjects had goitre.

Maurer and Diez (1926) show a rapid decline in I content of colostrum from 24 μg. on the 1st day *post partum* to 2·1 μg. on the 6th day, and a tendency to rise thereafter. Elmer and Rychlik (1934) also showed a decline in I during the colostral period (Fig. 7), and found a tendency for I in the milk to be inversely proportional to the volume of milk secreted.

Some at least of the high values recorded by Turner and Weeks were associated with the use of iodised salt. In view of the effect of I intake, differences and trends will be difficult to establish without careful control of diet.

Zinc

Birckner (1919) gives the level of Zn in negro milk as rising from 0·57 to 1·38 mg. per 100 ml. from the 4th to the 15th day *post partum*. Sato and Murata (1932) found the Zn content of colostrum to be higher than that of milk and stated that during most of lactation the level is about 0·2 to 0·3 mg. per 100 ml. Koga (1934) also reported that the Zn content of colostrum was higher than that of milk and that the Zn content of human milk was less than that of cow or goat.

Sulphur

For 4 samples of human colostrum Lucca and Lagani (1934) record a mean S of 0·139 per cent. and for 12 samples of milk 0·104 per cent. Révol and Paccard (1937) give the S content of human milk as varying between 8·2 and 20·2 mg. per 100 ml.

No information on fluorine or cobalt has been found.

SUMMARY

Yield. The techniques used to assess milk yield are not satisfactory. An infant need not and often does not remove all the milk and both hand and pump milking may affect milk yield. Hence all data on yield are, to some extent, suspect. Much of the data, and especially the more extensive series, comes from wet nurses and even between them individually the differences are very large. For these and other reasons only tentative conclusions can be reached on any of the questions raised.

There may be a difference in yield between breasts which may depend on difference in size. When yield is computed per hour so as to eliminate difference in interval between milkings, there is no real evidence for a diurnal rhythm. Yield increases from the start of lactation for an uncertain time to a plateau of uncertain duration and a slow decline. It is impossible from the data to distinguish the only partially associated effects of age and parity of mother, or of age and weight of child.

Only one study of the effects of varying diet within ordinary limits was found suitable for analysis ; the effects were slight. One study showed that milk yield is maintained against even severe reduction of water intake by economy of excretion by other routes. Excess fluid intake had no effect.

Nitrogen. The mean protein content of 915 single samples of mature milk, each from a different donor was 1·316 g. per 100 ml., S.D. 0·321. There is no definite evidence of any difference between the milk from the two breasts, nor of a change during a nursing or during a day. It is fairly well established that N content decreases during the first 10 days, the colostral period, and possibly longer. There may be a continued slow decline through most of lactation. Individual variation is considerable and so the mean picture of any small series of observations may be greatly modified by the characteristics of a single subject.

There is some evidence for a decrease of total N with parity and age, but confirmation is needed from larger numbers of repeated observations on the same women at the same stage of

different lactations. The evidence for an effect of level of protein in the diet is not consistent, but that from the largest single study suggests an increase in milk N with the protein of the diet.

In spite of a lot of work the partition of N remains doubtful on account of uncertainties in the interpretation of results with different methods of analysis. Electrophoretic methods may help. Agreement is better for amino-acids in milk protein and the results are tabulated in detail.

Fat and Lipoid Matter. The mean value for fat in 299 whole-day samples was 3·33 g. per 100 ml., S.D. 0·57. Most, but not all, recorded data show a diurnal rhythm with a maximum during the forenoon. Since the cause is unknown it is impossible to say whether the rhythm is spontaneous or induced. Most, but not all, show an increase of fat content from fore to after milk. There is no evidence of a uniform change with stage of lactation, volume, age or parity. Day to day variation may be great and the only clear evidence for an effect of diet comes from an experiment in which a supplement of 200 g. glucose raised the fat content of milk by 0·5 to 1·0 g. per 100 ml.

Recent data for the fatty acid composition are tabulated and differences from cow's milk fat are discussed. What information there is on non-fat lipids is reviewed.

Lactose. The mean value for 1010 samples was 7·23 g. per 100 ml., S.D. 0·67. Day-to-day variation is great but there is no definite evidence of any regular periodic change or effect of diet.

Inorganic Constituents. Total ash decreases rapidly at first and slowly and steadily in mature milk. The mean values recorded vary from 188 to 202 mg. per 100 ml.

Most recorded means for calcium lie between 28 and 30 mg. per 100 ml. but means vary from 22·4 to 34·4 mg. There is no significant diurnal or day-to-day variation and results for fore and after milk are contradictory. Mature milk shows a maximum about the third month. The effect of diet has not been sufficiently studied.

For total phosphorus, means vary from 13·0 to 17·54 mg. per 100 ml., most of them about 14 mg. Partition is discussed. There is no significant change, diurnal or during a nursing ; there is a slow decline through lactation but no other definite change.

Magnesium averaged 3·3 mg. ; chlorine 37·5 to 54·0 mg. with much variation and a rise at the end of lactation ; sodium 17·2 mg. in only major series ; potassium 51 to 52 mg. per 100 ml. Such evidence as there is for periodic change is reviewed. The sparse information on iron and copper, manganese, iodine, zinc and sulphur is discussed.

It is clear that there is plenty of room for work on every constituent. This review may serve to show where special care is needed in sampling technique and the spacing of sampling.

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